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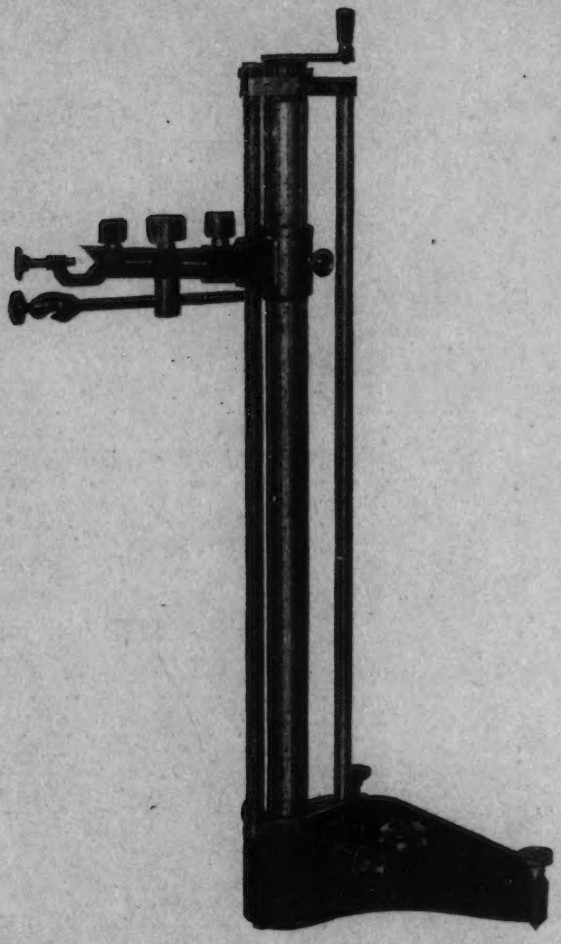

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THE NATURE OF THE STIMULUS WHICH EXCITES THE BLUE ARCS OF THE RETINA AND RELATED PHENOMENA

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From the author's laboratory in Newton Centre, Massachusetts

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In a recent article in this Journal (1927) I described the entoptic phenomena which result from secondary stimulation of the retina without any discussion of the nature of this secondary stimulus. The blue arcs and oval occasioned by this stimulus are so constantly found and so easily observed that they might be usefully employed in a number of physiological investigations if their cause were indisputably determined. It has been proved that, when a patch of light falls on the macula of a subject in a dark room, it produces a secondary agent which excites in a peculiar manner some of the papillo-macular nerve-fibers, or their subjacent receptors, giving rise to projected images of a grayish-blue color, at times tinged with a little red. These images are of two, and possibly of three kinds. For the purposes of this paper they will be referred to as the *arcs*, which are usually seen unless the primary stimulus is weak, and the *haze* which may cover the oval space between the arcs; I shall allude to this space hereafter as the *oval*. Attentive observation will frequently detect a halo of faint light about the primary image on the macula. This has been called the glow by Mrs. Ladd-Franklin, but I prefer to distinguish it as the *halo*, as it completely envelops the primary image, and as it is necessary to consider it apart from the haze which occurs in the oval. It is most probable that a part of this halo is due to the optical imperfections of the eye. A photograph of a small spot of light made with a simple, uncorrected lens has a similar halo. As a bluish color predominates in the entoptic halo we are authorized to infer that a sensation similar to that of the haze is superadded to that occasioned by the scattered light about the primary image.

Until recently the only theory of the nature of the secondary stimulus which excites the arcs that has received serious attention was that of Gertz (1905) who ascribed them to the electric disturbance originating in the

nerve-fibres connected with the receptors which are affected by the light of the primary image on the macula. It was assumed by him that the electric currents in these fibres extend to neighboring visual elements, and, by the stimulation of these elements, give rise to the perception of the arcs. Dr. Christine Ladd-Franklin (1927) does not accept this theory, but for several years has advocated another in which she assumes that the retinal nerve fibres excited by the macular image become luminescent, and that the radiation of their light stimulates the receptors which are located near them. Her arguments for the adoption of her theory are based largely on the supposed inadequacy of the electric hypothesis to explain the blue phenomena. She believes that all non-medullated nerve fibres radiate a certain amount of light during their excitation. It is evident that, if such a luminescence exists, it should be possible to demonstrate it objectively.

As the retina of the frog is quite similar in structure to that of man, and as it retains its vitality for a considerable time after the eye has been removed from the body, it would seem to be a very appropriate object for experimentation to test the luminescent theory. During the summer of 1927 I made numerous experiments with the eyes of frogs in a dark room, but I was unable to obtain any indication of luminescence of the stimulated retina. The methods employed should have revealed a slight amount of luminescence if it had been present. The frogs used had been recently caught, and were vigorous specimens. In one series of experiments I proceeded as follows. The eye was removed as soon as possible after the animal was decapitated. The posterior half of the bulb was separated from the anterior with a razor and with scissors, and was then placed in a special apparatus on a shelf supported on a tripod stand. The scleral surface of the hemisphere was placed in contact with a horizontal loop of aluminum wire which was connected with one pole of the secondary of an induction apparatus, and the other electrode was a perpendicular platinum wire with its lower end on the center of the retina. A 75 mm. lens, in a convenient holder which could slide on the tripod stand, was accurately focused on the retina. The observations were made in a room which was completely darkened. Stimuli of graded intensity were furnished by an ordinary induction apparatus. There were no indications of any luminescence either before or after stimulation with induction shocks applied for several seconds. The experiments were frequently varied by removing the retina immediately after the enucleation of the eye, and placing a portion of it, with the nerve fibre layer upwards, across the platinum tips of ordinary stimulating electrodes. The handle of the electrodes was held in a clamp on the tripod stand, and the strip of retina was observed through the lens in the dark room, and with entirely negative results, when it was stimulated with induction shocks. The observations were repeated many times, and the intensity of the stimuli varied from weak to very strong.

Mrs. Ladd-Franklin bases her chief objection to the electric theory of secondary retinal excitation on the contention that the sensation due to electric stimulation of the visual organ is never followed by an after-image, and also on the assumption that the arcs are followed by such an image. I have never been able to see any true after-image of the arcs although I have studied the after-images which have occurred in my experiments with great care, and under favorable circumstances. It is easy to observe a well-marked positive after-image of the halo, and a faint, bluish-gray after-image of the haze in which the situation and shape of the arcs are indicated by two dark defects. In other words, if there were no after-image of the haze there would probably be no indications of after-images of the arcs.

In order to study the after-images of the blue phenomena I have usually arranged the apparatus as follows. A photographer's dark-room lantern is closed in front with a piece of thick, dark red paper. In front of this light filter is a screen of black cardboard with a slit in its center, 5 mm. wide and 50 mm. long. The slit is illuminated indirectly by a tungsten bulb in the upper part of the lamp. The room has no window, and it is completely dark. After a few minutes in this room the eyes become sufficiently sensitive to observe all the after-images that occur. To assist in keeping the eye fixed a very small hole is made in the cardboard screen opposite the middle of the slit, and about 10 mm. from it, and this hole is viewed at a distance of 50 to 75 cm. The hole is first fixed with the eyes, then, without changing their direction, they are both covered. The observing eye, which is the one opposite the hole, is then uncovered for about two seconds, and then covered again. The indistinct, bluish-gray after-image of the haze usually lasts two or three seconds. In addition to this image a positive or negative image of the slit is seen surrounded with the after-image of the halo, which should not be confounded with that of the haze. The after-image of the halo is usually well marked, and may be a reddish-gray, or it may at times be tinged with another color. As we believe that a great part of this image is due to diffused light from the slit which should act upon the receptors in the vicinity and produce the ordinary after-images which follow stimulation by light, we shall not consider it further in this connection. The after-image of the haze has much the appearance of the ideoretinal light, but it is more intense and transitory.

The absence of true after-images of the arcs renders Mrs. Ladd-Franklin's chief argument for her view that the arcs are caused by visible light without force. As the arcs are the typical, and, by far, the most intense of the results of secondary stimulation, the absence of after-images of them goes far to disprove her theory, if the assumption upon which she largely bases that theory be true.

The second argument that Mrs. Ladd-Franklin offers to disprove the

electric theory of Gertz is that the sensations resulting from secondary excitation of retinal nerve fibres would not have the right "place coefficients." We assume that she means by the expression place coefficient the situation in the projected field of the sensation which would result from the activity of the receptor of which the stimulated fibre is the continuation. If it were only a question of locating the sensation due to one fibre there would be some validity in her argument, but what is seen is not a point of light, but two extensive luminous bands due to the stimultaneous stimulation of all the nerve fibres originating in the corresponding retinal region, or of all their receptors. It must be borne in mind that the bands of nerve fibres corresponding to the arcs contain, not only the fibres coming from the macular image, but many fibres belonging to the receptors beneath those bands. So far as the perception of the arcs is concerned it amounts to the same thing whether the receptors of the bands are primarily stimulated, or their nerve fibres, for the stimulation of all the fibres would locate the perception in the same place as the stimulation of all the receptors.

Electricity is the only agent which may act as a secondary stimulus which we know results from the primary stimulation of the retina with light. The experiments of Dewar and McKendrick (1875), and of subsequent investigators, have proven that, when the retina is so stimulated, the visual elements involved have their electric potential raised. Records obtained with the string galvanometer show that the first effect of the illumination is a very brief negative variation followed by a more extensive rise in potential, which may diminish to some extent, but remains elevated during the persistence of the stimulation. This result, originally observed in animals, has been obtained with the human subject by Hartline (1925). Although the records secured with different animals, and even, at times, with the same animal, vary, it has been firmly established that the predominant electric effect of the illumination of the eye is an increase in potential in the affected part of the retina. As all the tissues of the retina are conducting, the increase in potential in the portion of the retina primarily affected should produce electric currents. If these currents are of sufficient intensity they may affect other parts of the retina to which they spread, and occasion a characteristic sensation. It is known that stimulation of the eye with a galvanic current produces a sensation of a pale violet light. The color of this light corresponds closely to that of the arcs, and suggests that the arcs are the result of the same kind of stimulus. It has been found in the frog's eye that the removal of the light stimulus produces a second rise in its electric potential. It is not certain that this always occurs in warm-blooded animals, but there is in them, no doubt, an electric readjustment which may act as a second stimulus, and may possibly give rise to the appearance of an after-image. This second stimulus may explain the

occurrence of the faint after-image of the haze in our experiments, and the defects in this after-image which correspond to the arcs are possibly due to the effect of the more intense previous stimulation in the regions which they cover. It has been proven (Ellis, 1901) that, even after slight previous stimulation, visual elements may be refractory for a time to subsequent feeble stimulation. The haze is no doubt due to the same kind of stimulus as the arcs, but one of much less intensity, and one that is much more diffused. The after-image of the haze is so similar to the ideoretinal light that we have reason to suspect that they both have a similar origin. Experimental facts and theoretical considerations combine to render the electric theory of the causation of the arcs and haze a very probable one, and in the present state of our knowledge of the subject we may retain it as a working hypothesis.

SUMMARY

An account is given of experiments with frogs eyes to test Mrs. Ladd-Franklin's theory of the luminescence of stimulated nerve fibres. The results of these experiments were negative.

The after-images which follow the appearance of the blue arcs of the retina and their related phenomena are described, and their probable causes are stated.

The conclusion is drawn from the author's experiments that the electric theory of the causation of the blue arcs and haze of the retina is a most probable one.

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A DIENCEPHALIC MECHANISM FOR THE EXPRESSION OF RAGE WITH SPECIAL REFERENCE TO THE SYMPATHETIC NERVOUS SYSTEM

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At various times "the seat of the emotions" and the central mechanisms responsible for emotional behavior have been sought in the cerebral cortex. While it is reasonable to suppose that the neural processes underlying emotional consciousness are indeed cortical, it does not follow that the bodily changes which make up emotional behavior are due to a nervous discharge of cortical origin. The behavior attending the major emotions, fear and rage, is called forth by the urgency of certain definite circumstances and it is plainly directed toward the preservation of the individual. It constitutes a reaction which is primitive, energetically purposive and common to the divergent members of the vertebrate series. This consideration certainly suggests that the reaction is dependent upon older divisions of the nervous system. There is considerable evidence to show that this is actually the case.

Not only may the expression of one strong emotion, namely, anger, occur in the mammal deprived of its cerebral hemispheres, but in such an animal the tendency to exhibit this emotion is especially marked. The affective behavior of Goltz' dog (1892) was confined to a reaction, capable of regular elicitation, which closely resembled the rage of a normal dog. The same was true of the dog described by Rothmann (1923) and of the two hemisphereless cats prepared and studied by de Barenne (1920). The trivial and often irrelevant nature of the conditions which would evoke the reaction in these animals is noteworthy. A display of anger could be produced in Goltz' animal by pinching the skin or by taking it from its cage. The latter procedure invariably caused a violent protest in spite of the fact that it was the usual signal for feeding and would have been quite agreeable to a normal dog. In Rothmann's dog snarling and growling were obtained by gentle scratching of the back, and the presence of a fly on the creature's nose sent it into a fit of rage. De Barenne reports that his cats showed a similar emotional response to various disturbances, innocuous as well as painful; merely lifting them caused energetic movements of defense and those reactions which are so characteristic of the angry cat—spitting,

growling and erection of the hair of the back and tail. The picture presented by these animals implies the unrepressed activity of certain primitive subcortical mechanisms.

From the earliest times it has been recognized that emotional excitement is accompanied by profound visceral changes, and Cannon (1915) has stressed the fact that in fear and rage the viscera are dominated by a discharge of impulses over the sympathetic division of the autonomic system. Although this aspect of emotional behavior failed to receive any attention in the observations of Goltz, Rothmann and de Barenne, the knowledge that cortical ablation renders the mammal excessively prone to a display of anger led Cannon and Britton (1925) to use a decorticate preparation in the study of one important consequence of the emotional activation of the sympathetic, namely, medulliadrenal secretion. In acute experiments on cats they found that after disconnecting the cortex from the brain stem there appeared upon removing the anesthetic "a group of remarkable activities such as are usually associated with emotional excitement—a sort of sham rage." A prominent feature of this quasi-emotional state consisted of signs of widespread sympathetic activity; it was attended by erection of hair, profuse sweating from the toe pads, a high arterial pressure and, provided the adrenal glands remained intact, notable increments in the rate of the denervated heart. Bulatao and Cannon (1925) described the high glycemic percentages associated with the sham rage and showed that medulliadrenal secretion plays a major rôle in their production.

A hint as to the locus of the subcortical level responsible for this emotional activity of decorticate animals can be gained from the results of experiments in which cerebral ablation has not been followed by such behavior. In 1904 Woodworth and Sherrington gave the name "pseudaffective reflexes" to certain responses, expressive of affective states, which they obtained in decerebrate cats (hemispheres and diencephalon removed) on stimulating an afferent nerve. Similar reflexes were noted by Bazett and Penfield (1922) in their chronically decerebrate cats; lashing of the tail, kicking, running, even biting, and, very occasionally, growling occurred when the animals were disturbed. It is, however, important to distinguish between the pseudaffective activity of these midbrain preparations and that of decorticate animals. In the former, as exemplified by the experiments of Bazett and Penfield, it consists of isolated items of behavior, never attains a general affective state and is typically brought forth by a stimulus which is connected with some habitual mode of response. And although in the experiments of Woodworth and Sherrington these reflexes, evoked by strong afferent stimulation, had a certain "width of coördination," it was pointed out that "they never amounted to an effective action of attack or escape." On the other hand the sham rage of the decorticate

animal in the chronic (Goltz, Rothmann, de Barenne) as well as in the acute (Cannon and Britton) condition is elicited by trifling disturbances of any kind, it is astonishingly intense and possesses a width and energy of expression that makes it unmistakably the counterpart of intense fury in the normal animal. The level responsible for this more general and more energetic quasi-emotional behavior must lie above the mesencephalon.

The central source of the sympathetic discharge is primarily important in a study of the neural basis for emotional expression. In this connection it may be said that a central representation of certain fractions of the sympathetic system can be discerned at several points along the cerebrospinal axis, especially in the medulla oblongata. It is not unreasonable to regard the vasoconstrictor center, the bulbar cardio-accelerator mechanism and that portion of the medulla involved in Bernard's sugar puncture as sympathetic mechanisms, for the activation of each results in a discharge of impulses over certain groups of preganglionic sympathetic neurones. But it has been pointed out by Cannon (1915) that, in accord with the extensive distribution of its fibers and their arrangement for a diffuse discharge, the sympathetic, unlike the other divisions of the autonomic system, tends to discharge as a whole. This consideration in itself implies the existence of some dominant central coördinating mechanism which when active will produce a discharge of impulses over the entire series of sympathetic connector neurones. Cannon has further emphasized that the conditions under which this widespread discharge occurs are those which demand a vigorous response to ensure the maintenance of an essential condition or even of life itself. Foremost among these are the major emotions, fear and rage.

The present investigation was undertaken with this point in mind and was directed toward the delimitation of the part of the brain stem responsible for the sham rage of the decorticate cat. It was felt that not only would this elucidate the neural basis for this emotion but might yield some evidence for the existence of that predominant central mechanism which is so clearly implied by the tendency of the sympathetic to discharge vigorously and as a unit under conditions of stress.

The experimental procedure which was adopted as being the simplest and most direct method of realizing this delimitation was that of ablation of varying portions of the brain stem after removal of the hemispheres.

METHOD. Directly after etherization a tracheal cannula was inserted and the carotids ligated in the neck. After laying bare a large area of the parietal and a small part of the frontal bones, a trephine hole was made to one side of the mid-line and enlarged so as to expose the dorsal surfaces of the two hemispheres. Diploic bleeding was arrested by the application of pledgets of cotton. The mid-line was not crossed until the opening on one side had been completed and, as a rule, hemorrhage was slight provided

care was taken not to injure the dura and underlying vessels. On passing across the mid-line the longitudinal sinus must necessarily be ruptured and therefore the rest of the operation was carried out with the greatest dispatch while an assistant compressed the vertebral arteries. After incising the dura the occipital pole of one hemisphere was raised so as to bring into view the posterior colliculus of the same side. A flattened and somewhat curved blunt dissector was inserted toward the base and brought to a position just lateral to the inferior quadrigeminal brachium. Holding the handle of the instrument toward the opposite side it was then possible by means of a single forward movement to remove the entire cortex and medullary center of one hemisphere. The procedure was repeated on the other side and this resulted in the ablation of the entire cortex except a small medial portion of each pyriform lobe. Immediately, the desired extent of brain stem was removed by making a frontal transection or, as in a number of experiments, by slicing away the dorsal parts of the diencephalon after removal of the corpora striata. A large sharp knife was used in order to produce a clean-cut surface with minimal traumatization of adjacent tissue. The cut surface was packed lightly with cotton moistened with warm Ringer-Locke's solution. When bleeding was persistent, small strips of muscle were applied. Then, after gradually releasing the pressure on the vertebrals, hemostasis was usually complete.

The entire operation was done as rapidly as possible. It was found that better results were obtained by working rapidly with vertebrals compressed than by proceeding slowly and attempting to check hemorrhage at each step. The respiration afforded a good index of the degree of cerebral anemia. As a rule the operation could be completed before any marked respiratory disturbance developed.

Since all parts of the cortex which subserve sensation, including pain, were removed, the ether was discontinued as soon as the cerebral ablation was completed. The development of the sham rage was thus made possible. The cat was placed on an animal board in the dorsal position and secured by all four feet with the head slightly elevated. While it was recovering from the anesthetic a femoral artery was cannulated and connected with a mercury manometer.

A detailed record of the behavior of the animal was kept throughout each experiment. Changes in the heart rate and arterial pressure were shown by the tracings; the respiratory rate was taken and all symptoms of significance were noted. These records and notations were made at varying intervals of time depending upon the changes in behavior. The temperature of the animals, as shown by a rectal thermometer, was maintained as nearly as possible within a degree Centigrade of the normal level. At the end of each experiment the remaining portion of the brain was carefully removed from the cranium, fixed in formalin and preserved in 70 per cent

alcohol. A careful gross examination of these brains was supplemented by a study of the frontal sections of the cat's brain shown in Winkler and Potter's (1914) book. In this way it was possible to determine the extent of the ablation with a reasonable degree of macroscopic accuracy. In addition an histological study, still incomplete, has been made of sections cut from a number of these brain stems.

During the initial stage of the investigation the pseudaffective state was first produced by decortication, and then later the brain stem was cut across in an effort to determine the level of transection necessary to abolish the activity. Experience taught, however, that a second intervention was hazardous, especially in the face of the high arterial pressure which accompanies the activity. Therefore, in general, the method adopted was to determine the behavior following a single definite ablation. In a few of the earlier experiments the vagi were cut in the neck and the stellate ganglia removed; in the acute experiment this denervation renders the heart an indicator of medulliadrenal secretion (Cannon, Lewis and Britton, 1926). But in the majority of the experiments the nerves were left intact.

Results. Records of behavior following cerebral ablation were obtained in 52 cats. Because of early death, hemorrhage or inadvertent injury of the remaining parts of the brain, 6 of these failed to be of any service in the localization of the region responsible for the sham rage. The following report is, therefore, based upon the results of 46 experiments.

The pseudaffective phenomena which occurred in these experiments were quite similar to those described by Cannon and Britton (1925). They developed spontaneously or could be evoked by the slightest disturbance; they usually appeared in fits which lasted from a few seconds to several minutes. They included struggling, attended by movements of the head and arching of the trunk with thrusting and pulling of the limbs; clawing movements of the fore legs with protrusion of the claws; waving and lashing of the tail; a snarling expression; and very rapid panting with mouth open and movements of the tongue to and fro. In addition to these activities were signs denoting a vigorous sympathetic discharge: erection of the tail hairs; sweating from the toe pads; retraction of the nictitating membranes; exophthalmos (separation of the lids); large increments in arterial pressure and heart rate. These activities sometimes appeared in incomplete combinations, two or three of them being absent during the whole or a part of an experiment. Pupillo-dilatation was noted in some cases, but since it may be brought about either by sympathetic activity (cervical sympathetic impulses or circulating adrenin) or by inhibition of the tonic discharge over oculomotor fibers supplying the sphincter of the iris, it cannot be taken as a certain indicator of the former. A similar difficulty is encountered in connection with the acceleration of the innervated heart. This might be attributed to a sympathetic discharge acting directly by way

of the accelerators or indirectly through humoral factors; on the other hand, it might be due to central inhibition of the tonic vagal discharge. No attempt has been made to determine the relative importance of these three factors, but in view of the generalized sympathetic activity under these conditions it is certain that accelerator impulses play a rôle in the production of these more rapid rates. Cardiac acceleration frequently occurred simultaneously with the rises in arterial pressure and during the fits of sham rage there was never the slightest tendency to obey Marey's law.

All experiments were acute. The length of life was variable; but, in general, the more active the animal the shorter the survival. One of the inactive preparations survived over 19 hours and was finally killed while still in good condition, but in the whole series, the average duration of life was 4 hours. It was found that whenever the true pseudaffective behavior (sham rage) made its appearance it did so within 35 minutes of the completion of the cerebral ablation. There was only one exception to this rule, experiment 21, in which the activity did not appear until 65 minutes had elapsed; but in this case a moderate hemorrhage intervened and this may have temporarily postponed the development of activity.

The testimony of all who have studied decerebrate cats is consistent in showing that the decorticate sham rage never occurs in these preparations. Decerebration is commonly carried out by transecting the midbrain at a level passing between the superior and inferior colliculi dorsally and the roots of the third nerves ventrally. Often, as in the majority of Bazett and Penfield's animals, the ablation is extended to the most caudal parts of the midbrain. In this enquiry, therefore, the exploration of the brain stem has been confined to the diencephalon, the cranial part of the mesencephalon and, especially, to the zone of transition between these two divisions. Winkler and Potter (1914) have been followed in considering as diencephalon that part of the brain stem, "which is limited frontally by the anterior commissure and the chiasma n. optici, and caudally by the epiphysis and corpus mammillare."

The sham rage occurs after ablation of hemispheres, corpora striata and the cranial half of the diencephalon. This was especially well demonstrated by seven experiments in which decortication was followed by frontal transections, at various levels, through the diencephalon. In each of these there occurred the same vigorous quasi-emotional behavior which Cannon and Britton (1925) produced by decortication. Since these experiments are especially important in the delimitation of the central region responsible for the behavior, they will be described in some detail.

Experiment 1. After removal of the hemispheres the brain stem was transected through the cranial part of the thalamic region; the section was frontal and struck the base at the chiasma. Twenty-four minutes later, at 2:47, the first activity ap-

peared quite spontaneously. It consisted of a fit of struggling with lashing of the tail, marked erection of the tail hairs, protrusion of the claws, snarling and a rapid rise in arterial tension from 130 to 160 mm. Hg. The denervated heart beat at a rate of 244 per minute. Four minutes later the animal was lying quiet; the hairs remained unruffled after being smoothed and the heart rate had dropped to 226. From 2:51 to 3:19 similar fits appeared at intervals. The tail hairs were erected with the onset of activity and they became smooth during the intervening periods of quiet. Similarly the arterial pressure and heart rate increased roughly in proportion to the activity and fell when the animal became quiet. During one intense struggle biting occurred while the head was thrown from side to side. At 3:19 the activity became continuous. From 3:19 to 3:26 the pads of the hind feet were moist with sweat.

At 3:27 a second transection was made, this time through the midbrain. (Dorsally it shaved the cranial borders of the superior colliculi and ventrally it passed just behind the roots of the third nerves.) Following this the animal remained quiet until its death at 7:05; the tail was still and the hairs down, the claws were never protruded, the pulse rate gradually fell from 288 at 3:26 to 186 at 6:53, and the arterial pressure remained nearly constant and above 90 mm. Hg until just before death. At 4:32 rough handling induced lashing of the tail which ceased as soon as the animal was left alone; there were no other reactions. This transection was followed by strong decerebrate rigidity.

Experiment 4. The only transection was made at the time of decortication. This passed more caudally than the first transection of experiment 1. It struck the base between the optic chiasma and the stalk of the hypophysis. For 35 minutes after removal of the ether the animal remained quiet and exhibited decerebrate rigidity. It then proceeded to show more and more spontaneous activity and for the next two hours presented much the same behavior as did the cat of experiment 1. There was struggling, lashing of the tail and protrusion of the claws. It failed to sweat, but the tail was bushy throughout; there were marked rises in arterial pressure (e.g., 112–152 mm. Hg) whenever activity occurred; the heart was denervated and its rate varied from 200 per minute during a long period of quiet to 254, the rate recorded at the time of maximal activity. During the quiet periods the most intense sham rage could be induced simply by loosening the thong binding one of the legs. Toward the end of the experiment, as the spontaneous activity became less and less, the extensor tone of the limbs became conspicuous.

Experiment 26. Transection at mid-thalamic level; the cut passed from a point 3–4 mm. in front of pineal body to the middle of the tuber cinereum. Heart not denervated and sympathetic innervation of eyes left intact. From 1:54, the time of the transection, until 2:29 slight passive movements evoked struggling, protrusion of claws and rises in arterial pressure (e.g., 110–144 mm. Hg), but eyes remained closed and covered with relaxed membranes; there was no sweating. At 2:29 there was a strong brief and spontaneous fit of activity: struggling, protrusion of claws, simultaneous rises in heart rate (296–302 per min.) and arterial pressure (112–138 mm. Hg). At this time sweat appeared on the toe pads. Similar periods of activity, more prolonged and frequent, appeared up to 4:00. During one intense fit the head was thrown from side to side and the mouth opened in a typical snarl. During the few periods of quiet the eyes were closed and the nictitating membranes relaxed, but with the onset of activity the palpebral space was widened and the membranes completely withdrawn; similarly the arterial pressure diminished and remained constant. From 4:00 to 5:30 the animal remained quiet unless disturbed, when a typical strong fit of activity immediately developed. Sweating ceased at 3:26. The respiratory rate was above normal from the first, and typical panting set

TABLE 1

(*Experiment 34*)

TIME	RECTAL TEMPERATURE	HEART RATE PER MINUTE	ARTERIAL PRESSURE	RESPIRATIONS PER MINUTE	NOTES
			mm. Hg		
2:05					Decortication and transection completed; ether off. Heart not denervated
2:24	36.3	170	162	40	Quiet; hairs down; claws in; eyes closed; pupils moderately dilated; nictitating membranes far over eyes; rigidity
2:37	37.2	198	180	44	Tail waving; hairs erected; head movements; eyes half open; membranes partly retracted
2:42	37.3	178–216	210–240	52	Slight spontaneous struggle; head moved; hairs further erected; tail still. Bending neck caused the rises in arterial pressure and heart rate recorded
2:47	37.2	174	175	34	Quiet; hairs erected; claws in; eyes half closed; pupils much wider; membranes far out over eyes; rigidity
2:57	37.2	220	208–222	28	Spontaneous struggle; tail waving
3:05	37.3	192	185	30	Quiet; hairs slightly erected
3:20	37.3	186	164	28	Quiet since 3:13. Tail still; hairs same; eyes closed; membranes far out over eyes; pupils wide; claws in; rigidity
3:28	37.2	204	185±	30	Spontaneous struggle with rise in arterial pressure; further erection of hair; opening of eyes; retraction of nictitating membranes; claws in
3:47	37.1	192	169±	32	Continuous spontaneous struggle; claws out; tail waving; hairs up; eyes wide; membranes retracted; sweat on toe pads
3:56	37.0	200±	118–133	26	The same
4:05	37.1	194	137	34	The same
4:20	37.1	228–240	122–140	34	Spontaneous struggling at intervals; claws out; tail waving; hairs up; sweat on pads
4:37	37.3	210–216	138–160	44	Tapping leg with pencil causes struggle with rises in heart rate and arterial pressure; widening of eyes and retraction of membranes. Claws out; pads damp
5:24	36.7	183	134–160	36	Slightest touch evokes activity as before; pads damp
5:30–11:28	37.6–36.5	242–196	115–53	56–34	No activity either induced or spontaneous. Arterial pressure gradually fell; above 88 till 7:45
11:54					Killed

TABLE 2

(*Experiment 13*)

TIME	RECTAL TEMPERATURE	HEART RATE PER MINUTE	ARTERIAL PRESSURE	RESPIRATIONS PER MINUTE	NOTES
			mm. Hg		
2:26					Decortication and transection completed; ether off. Heart not denervated
2:39		302	136	172	Quiet; hairs up slightly; panting; claws in; rigidity
2:44	37.0	228	128	240	Same; tail waving
2:48	37.0	244	120–144	216	Spontaneous struggle; hairs further erected; claws out; panting slower and deeper with struggle
2:54	37.0	256	110–128	200	Vigorous spontaneous struggle; tail lashing and bushy; claws maximally protruded; movements of vibrissae; snarling expression; panting tongue to and fro
2:58	36.8	256–264	102–130	240	Continuous activity, more intense; eyes very wide
3:03	36.8	256–268	116–144	200–240	Continuous maximal activity; pulling and tugging; claws maximally protruded; tail lashing and bushy; arterial pressure and heart rate up and down with activity
3:11	36.7	262	87–100	204	Slight struggle; tail waving and bushy; claws out; panting with mouth open and tongue to and fro. Sweat on toe pads
3:15		256	83	232	Continuous activity; but not so intense as at 3:03
3:17	36.5	246–256	74–90		Intense struggling from moment to moment; more moderate activity during intervals; no periods of quiet; no rigidity
3:22	36.3	246	55–62	160	The same; pupils and eyes very wide; pads moist
3:28	36.2	244	65	170	Quiet followed by one slight struggle with protrusion of claws. Wide pupils; panting; continuous waving of tail and hairs erected after smoothing down; rigidity
3:31	36.2	246	65	180	Strong struggle with increased respiratory rate; movement of vibrissae; no rigidity
3:40–3:45	36.0	236	44	200	Struggling from time to time; claws maximally protruded; tail waving and bushy; pupils very wide; panting at increased rate, mouth open and tongue to and fro
3:55					Cat died; respiratory failure and falling arterial pressure

in at 2:50, attaining a rate of 160 per minute toward end of experiment (5:30). Animal lacked tail; no observations on hair.

Experiment 5. Same ablation as in experiment 26 and showed essentially the same behavior.

That the sham rage will develop after a still more caudal truncation of the brain stem is shown by experiments 34 and 13. Figures 1 and 2 indicate that in cat 34 the transection struck the base a trifle behind the level represented by experiment 26, but dorsally it is distinctly more caudal. Reference to Winkler and Potter (1914) shows that the distal half of the thalamus, a corresponding portion of the hypothalamus and a small portion of the geniculate bodies remain in the diencephalon. The corpora striata were completely removed. The behavior of this animal, described in detail in table 1, was intensely quasi-emotional. Besides the changes involving skeletal muscle there were the usual signs of a vigorous and widespread sympathetic discharge. In this experiment, as well as in experiment 26 and all which follow, the heart was not denervated. Accordingly, with stellate ganglia and cervical sympathetic trunks intact, widening of the eyes, retraction of the nictitating membranes and sweating from the fore feet were among the sympathetic symptoms which appeared during the active periods. In this instance sweat appeared on the toe pads of all four feet, but only after prolonged activity. In almost every case in which this symptom appeared it was characterized by a certain latency and it usually disappeared before the other signs of sympathetic discharge abated. In experiment 13, the brain from which is shown in figures 3 and 4, the transection passed in the same frontal plane as in 34, but the level is about a millimeter more caudal. Nevertheless, as indicated in table 2, this cat spontaneously exhibited all the signs of extreme rage. It was the most intense activity encountered in the entire investigation and it continued with only one brief interruption for over an hour. Its vigor brought about an early death from reactionary hemorrhage. This was the most caudal truncation of the brain stem that was followed by a spontaneous exhibition of the pseudaffective state with its full complement of activities.

Experiment 33 is of some special interest. Here the transection was made at about the same level as in the two experiments just described, but it ran a markedly oblique course. Judged by experiments 34 and 13 enough tissue to the right of the mid-line was spared to permit the development of the sham rage, but at a point 2 mm. to the left of the mid-line the cut attained a level which other experiments have shown produces a quiet decerebrate animal. In spite of this somewhat unilateral ablation, table 3 reveals the fact that for over 7 hours the cat exhibited the activity typical of sham rage. And it may be added that there were no indications of any differences in the motor activities of the two sides of the body.

Observations of the behavior following transections of the diencephalon

TABLE 3

(Experiment 33)

TIME	RECTAL TEMPERATURE	HEART RATE PER MINUTE	ARTERIAL PRESSURE	RESPIRATIONS PER MINUTE	NOTES
			mm. Hg		
2:01					Decortication and transection completed; ether off. Heart not denervated. No artificial heating till 5:00
2:19	38.8	166	165	28	Quiet; tail bushy; claws in; pads dry; eyes closed; pupils narrow; nictitating membranes out; rigidity
2:29	38.7	178	174	20	Tail lashing, bushy; claws out; eyes open; membranes retracted
2:35	38.5	188	170	28	The same
2:42	38.4	192	154–180	18	Spontaneous struggle with rise in arterial pressure; claws out; tail lashing, bushy; eyes wide; membranes retracted
2:49		198–228	158–177	26	No struggle; otherwise the same. (Release of leg induces struggle with increase of heart rate and arterial pressure)
2:58	38.2	174–210	150–156	22	Strong spontaneous struggle with rises in arterial pressure and heart rate; claws out; tail lashing, bushy; eyes wide; membranes back; sweat on toe pads
3:04	38.4	186	115	28	Quiet; tail quiet, bushy; otherwise as before
3:13	38.3	192	118	24	The same
3:29–3:40	38.3	198–202	138–158	28	Tail waving, bushy; claws maximally protruded; eyes wide; pupils very wide; membranes slightly out; pads moist; slightest touch evokes intense activity
3:50	38.2	202–222	133–150	24	Spontaneous struggle with rises in arterial pressure and heart rate; tail lashing; claws out
4:00	38.0	202	123	24	Quiet; intermittent waving of tail, bushy; claws out; eyes half closed; membranes over eyes; membranes quickly and completely retracted on releasing leg
4:08	38.2	204–240	125–156	20	Quiet; release of foreleg causes struggle, waving of tail, further erection of hairs, fresh retraction of nictitating membranes, definite dilatation of pupils, rises in arterial pressure and heart rate
4:30–4:48	37.8	210–232	130–170	30	Quiet; tail still but bushy; claws out; rigidity; moving leg or thermometer evokes struggle, tail waving; further protrusion of claws, separation of lids, abrupt retraction of membranes, simultaneous rises in arterial pressure and heart rate

TABLE 3—*Concluded*

TIME	RECTAL TEMPERATURE	HEART RATE PER MINUTE	ARTERIAL PRESSURE	RESPIRATIONS PER MINUTE	NOTES
			mm. Hg		
5:00	37.6	230	144	34	The same. Heating pad applied for first time
7:25	38.5	210–240	117–187	40	(Next observation.) Intense spontaneous struggling from time to time with rises in arterial pressure and heart rate as indicated, lashing of tail, further protrusion of claws and erection of tail hairs, retraction of nictitating membranes, opening of mouth and snarling. Panting; mouth open, tongue to and fro
7:38	38.6	222–240	118–160	40	The same intermittent spontaneous activity
9:01	37.7	240	124–158	56	The same. Pads dry
9:16–9:25	37.6	238–248	100	36	Quiet; tail still but bushy; claws out; eyes half closed, membranes over eyes
9:43	37.5	248	86	28	Brief spontaneous struggle; slightest disturbance evokes activity
10:22	38.0	252	68	26	Quiet since 9:43, but tail bushy and claws partially protruded. Rigidity. Cannot induce any activity by manipulating animal
10:30					Killed

between the levels represented in experiments 1 and 34 were obtained in six other animals. In each of these the typical activity made its appearance and ran the same general course as in the seven experiments detailed above. In the light of the results obtained when more caudal transections were made, the appearance of the sham rage in thirteen out of a total of thirteen experiments is a result of outstanding significance.

The sham rage fails to develop after transecting the caudal extremity of the diencephalon or the cranial portion of the mesencephalon. Because of a certain interlocking of diencephalon and mesencephalon in the cat's brain, it is not possible to divide one from the other along any frontal plane. Yet a transverse section which passes downward from the cranial borders of the superior colliculi to reach the base just behind the mammillary bodies will separate them approximately (Winkler and Potter, 1914). Cranial to it will lie the entire hypothalamus except the distal extremities of the corpora subthalamica which are about to give place on each side to the substantia nigra. Behind it there will remain a small caudal portion of the ventral and posterior nuclei of the thalamus and the greater mass of the geniculate bodies which embrace the mesencephalon dorso-laterally.

On the other hand this section will just cut off the cranial ends of the mesencephalic red nuclei which extend forward between the nuclei of the hypothalamus.

A number of experiments were devoted to the exploration of this zone of transition between diencephalon and mesencephalon. In none of them did a true decorticate sham rage develop, nor could it be induced by disturbances which invariably evoke it in animals retaining the caudal half of the diencephalon. Protocols of some of these experiments follow.

Experiment 52. Frontal transection made at 10:02 a.m. Figures 5 and 6 show that it passed through the pineal body dorsally and cut away the cranial two-thirds of the mammillary bodies ventrally. Animal observed for 6 hours. Remained in excellent condition, arterial pressure averaging around 95 mm. Hg. Showed decerebrate rigidity and no spontaneous activity of any kind. Respiration suddenly became rapid at 11:40 and remained around 150 per minute thereafter; this high rate persisted after reducing body temperature from 38.0° to 35.0°C. Mouth kept closed and no true panting. Moving (or inserting rectal thermometer) caused brief struggling, with tail waving, slight protrusion of claws, moderate increments in arterial pressure (e.g., 94–106 mm. Hg) and heart rate (e.g., 202–220 per minute). Moving limbs or whole cat or pinching evoked nothing. Tail hairs became slightly erected with the induced activity, but no other signs of sympathetic activity appeared; nictitating membranes remained out over partially closed eyes; there was no sweating.

Experiment 40. Brain shown in figures 7 and 8. Transection struck base through caudal third of mammillary bodies; above, it passed more cranially than in experiment 52. Animal killed in good condition after 19 hours of observation. During first five hours release of legs caused slow head movements and protrusion of claws with rise in arterial pressure and, occasionally, a small increase in heart rate—nothing else. During next nine hours only tail waving could be induced. For remainder of experiment moving legs passively brought on movements of progression. Toward end of experiment kicking and tugging with protrusion of claws followed application of hot-water bag; this was attended by huge rises in arterial pressure (e.g., 85–160 mm. Hg). This activity was not accompanied by any ocular changes, erection of hair or sweating. The pulse rate decreased as arterial pressure rose. Immediately after the cerebral ablation the heart rate was slow, 112 per minute, but it soon became more rapid and for the rest of the experiment vacillated back and forth between 160 and 212; no marked accelerations occurred at any time. The respiratory rate varied from 34 to 56 per minute. Strong decerebrate rigidity persisted throughout the 19 hours.

Experiment 51. A clean cut was made across the brain stem at 10:15 a.m. It passed down the cranial borders of the superior colliculi and severed the mammillary bodies through their caudal third. From 10:15 to 11:50 the animal showed no activity except very slight struggling with waving of the tail and trifling rises in arterial pressure (e.g., 138–145 mm. Hg) on moving thermometer in rectum. Only once did this cause cardiac acceleration (204–236 per minute). The claws were not protruded, the eyes were partly closed and the nictitating membranes remained relaxed and immobile; there was no sweating, but from the beginning the hair of the tail was slightly erected. Between 11:50 a.m. and 12:18 p.m. the central end of one crural nerve was stimulated electrically at three different times. It regularly produced waving of the tail, weak struggling, acceleration of the respiration (40–60 per

minute), erection of the tail hairs, abrupt retraction of the nictitating membranes, pupillo-dilatation and increased arterial tension, the maximal rise being from 115 to 175 mm. Hg; only once did cardiac acceleration occur (260–280 beats per minute). The pads remained dry and the claws were never protruded. The activity immediately subsided after the stimulation ceased. From 12:30 until the animal was killed at 3:00 p.m. it remained quiet except for weak waving of the tail from time to time. Rigidity was present in all four legs throughout.

The brain stem was transected at this level, i.e., at the caudal extremity of the diencephalon, in a total of seven experiments. In the other four the results were precisely the same as those described in these three protocols. At no time in any of these animals did there develop either spontaneously or as a consequence of stimulation a behavior which even approximated the picture of intense fury which regularly occurs after retention of the caudal half of the diencephalon. It is quite true that a few of the reactions which make up the sham rage were obtained in these cats by such disturbances as moving the limbs or rectal thermometer, but they were limited in extent and vigor. It is also wholly apparent that they lack the spontaneity and easy elicitation of the sham rage. Even the widespread reaction obtained in cat 51 by direct stimulation of an afferent nerve could not measure up to the fits of sham rage invariably seen after the more cranial transections.

In five animals the brain stem was cut across through the cranial part of the mesencephalon. In experiment 1, already described, such a transection put an end to the strong activity which had followed decortication and ablation of all of the brain stem cranial to the chiasma. Typical of these were experiments 46 and 31. Protocols of these follow.

Experiment 46. The transection shaved the cranial borders of the superior colliculi and reached the base just behind the mammillary bodies. The observation covered 4 hours and 35 minutes; it was terminated by killing the animal. No struggling, tail waving, widening of the eyes, retraction of the nictitating membranes or sweating occurred either spontaneously or in response to the usual disturbances. Tail hairs slightly erected throughout. Arterial pressure and heart rate were variable, but changes developed slowly and without apparent cause. On several occasions passive movement of legs induced moderate rises in arterial pressure curve (e.g., 93–115, 107–129 mm. Hg). Heart rate varied from 212 at beginning to 292 beats per minute at end. Respiration normal during first two hours; it then quickened and reached rate of 72 per minute, but there was no true panting. Decerebrate rigidity was present throughout.

Experiment 31. Brain shown in figures 9 and 10. Observed from 3:17 to 7:00 p.m. No spontaneous activity; slight waving of tail was the only symptom that could be evoked by moving the legs or rectal thermometer. Claws never protruded, mouth closed and no panting. Respiratory rate increased from 33, at beginning, to 58 per minute at end. Rigidity was present throughout. Tail hairs very slightly erected, eyes remained closed and covered by relaxed nictitating membranes. No sweat. TraubeHering waves appeared in pressure tracing; there was a gradual fall in arterial pressure from 120 mm. at 3:38 to 76 mm. at 5:53, and finally to 62 at 7:00. Until 5:15

the heart rate varied between 132 and 148 per minute; at 5:53, in the absence of any other bodily changes, it mounted quickly to 228 per minute and remained there to the end of the experiment. Strong rigidity of the limb extensors persisted throughout.

The sham rage may occur after ablation of the dorsal parts of the diencephalon. The experiments cited thus far have shown that if the caudal half of the diencephalon remain connected with the lower divisions of the brain stem a stage of sham rage will develop. They fail to show specifically whether the part essential for the activity is located dorsally or ventrally in this region. The apparently well-established relation of the hypothalamus to the sympathetic system suggested that the basal part of the diencephalon might be the source of this quasi-emotional behavior with its sympathteic discharge. Therefore in a large number of animals an attempt was made to leave only the hypothalamus connected with the midbrain. While twelve cats survived this more difficult operation for periods of sufficient duration to determine whether the sham rage would develop, only four exhibited it. A microscopic examination of their brains has shown that the desired separation was never wholly effected; in each a little of the ventral and caudal parts of the thalamus remained intact. Nevertheless these experiments are of some service in attaining a more precise delimitation.

Experiment 44 represents the most extensive dorsal ablation which was followed by the sham rage. A lateral view of the brain from this cat is presented in figure 11. A microscopic study of serial sections cut from it has shown that there remained in the diencephalon all of the hypothalamus which lies behind the chiasma except the cranial ends of the corpora subthalamica, a thin lamina of the ventral part of the thalamus, and all of the caudal extremity of the thalamus except the pulvinar. Table 4 gives some impression of the intense quasi-emotional behavior of this animal. The visceral changes which were such a prominent feature of the activity can leave no doubt as to the general involvement of the sympathetic system. The extraordinarily high rate of the innervated heart, the profuse sweating and the marked ocular changes warrant special notice.

Similar ablations were made in experiments 21 and 37, and each gave positive results. Again, histological examination of the brain remnants from these animals revealed small ventral and caudal fractions of the thalamus and the greater part of the hypothalamus. In both animals vigorous struggling occurred spontaneously and could be induced by the slightest disturbance, but for the most part the activity involving skeletal muscle was confined to a constant protrusion of the claws, incessant lashing of the tail and an occasional expression of snarling. These activities were accompanied by maximal changes in organs innervated by the sympathetic system. In short there seemed to be a preponderance of

TABLE 4

(Experiment 44)

TIME	RECTAL TEMPERATURE	HEART RATE PER MINUTE	ARTERIAL PRESSURE	RESPIRATIONS PER MINUTE	NOTES
			mm. Hg		
9:50					Decortication, transection at level of chiasma and ablation of dorsal part of diencephalon
10:09	37.2	208	167	32	Quiet; hairs up; pads dry; claws in; eyes half closed; nictitating membranes over eyes; rigidity
10:18	37.2	232–248	182–194	28	Slight spontaneous struggle with rises in arterial pressure and heart rate; tail waving
10:22		262	184		The same; claws out continuously; fore feet wet with sweat
10:28–10:35	37.7	290–296	187–215	32	Continuous struggling; tail lashing and bushy; maximal protrusion of claws; fore feet dripping with sweat, hind pads moist; with struggling, eyes and pupils widen, membranes retract and heart rate and arterial pressure rise rapidly
10:45	38.0	308–320	160–189	44	Intense fits of struggling accompanied by tail lashing, protrusion of claws, widening of eyes and pupils, retraction of membranes, rises in arterial pressure and pulse rate; tail bushy; fore feet dripping with sweat, hind pads moist
10:50	38.0	308	115–143	38	The same
11:05	38.0	300–308	90–106	40	Spontaneous struggling less frequent, but slightest touch induces maximal activity
11:20	37.8	310–318	90–122	40	Spontaneous activity as before. Sweating not so profuse
11:35	37.6	302	76–90	40	The same
11:55–12:45	37.0	296–300	95±	36–30	Quiet; tail bushy; claws out; pads dry; eyes partly closed; nictitating membranes relaxed
1:30	37.9	296–300	93–117	30	The same; passive flexion of leg induces struggle with tail waving, protrusion of claws, rises in arterial pressure and heart rate; slight rigidity
2:56	37.6	300	89–148	32	The same
3:27	37.4	290	76	30	The same; can induce activity by slightest disturbance
3:45					Killed

sympathetic over cerebro-spinal discharge. At the same time the character and coördination of the activity left no doubt in the observer's mind as to the type of behavior it represented; it was unquestionably a sham rage. The very absence of strong body and limb movements, except when the animals were disturbed, made the behavior of these animals very like that of a normal cat which is feeling savage. In both conditions the body and limbs are kept still, but the bristling hair, the ready claws, the lashing of the tail, the wide eyes and pupils afford unmistakable evidence of an intense affective state. In both cases a slight disturbance evokes vigorous movements of attack.

The inactivity of eight out of the twelve preparations which belong to this series is not difficult to account for. Examination of the brains from four of these negative experiments showed that the ablation had extended so close to the base over the mammillary bodies as to make them essentially midbrain animals. In the other four, however, the ablation was no different than in experiments 44, 21 and 37. The reason for their inactivity may be due to the fact that the longitudinal slicing of the diencephalon plainly exerts traction on the brain stem and it is only too likely that this might disrupt the mechanisms which are essential for the development of the sham rage. But, whatever the explanation, these particular negative results may be legitimately disregarded; the really significant fact is that positive results were obtained after making comparable ablations.

These experiments make possible a still further restriction of the central region which must be left intact if the decorticate sham rage is to develop. The method of frontal transection showed that the behavior is dependent upon mechanisms which lie within the caudal half of the diencephalon, but in view of the results of these dorsal ablations it becomes apparent that it is the ventral and caudal parts of this half which are essential. This delimitation is illustrated in figure 12 which presents a sagittal view of the brain stem of a cat. The lines represent the extent of the ablation in three representative and important experiments; in 34 and 44 the sham rage developed; in 40 it failed to do so, and, as will be remembered, in no case did the typical behavior appear after sections at or behind this level.

The transitional type of behavior obtained after transecting the caudal part of the diencephalon. There remain to be considered the results of an exploration of that segment of the brain stem which lies between the levels of transection represented by experiments 13 and 52 (see pp. 499 and 502). It has been shown that the presence or absence of this part of the diencephalon determines whether the sham rage does or does not follow removal of the higher parts of the cerebrum. Successful transections were made through this region in 9 cats. Since some fraction of the essential part

remained in each, it is not surprising that 6 out of the 9 exhibited a type of behavior which was distinctly transitional between the sham rage of the decorticate animal and the relatively weak and less definitive reactions of the midbrain preparation. The activity of the remaining 3 was confined to reactions of the latter sort. Two experiments afford fair examples of this intermediate type of behavior. Ventral views of the brains from these cats are shown in figures 13 and 14.

Experiment 47. Transection at 11:00 a.m. It began dorsally at precisely the same point as in experiment 13 (see fig. 4) but ventrally it passed about a millimeter behind the level of that transection and struck the base at the cranial border of the mammillary bodies. On the left side lateral slicing removed part of the cerebral peduncle and some of the overlying tissue. This animal remained in excellent condition up to 5:40 p.m. when it was sacrificed, but showed no spontaneous activity. When undisturbed it remained motionless with tail hairs erected, claws in, eyes closed, nictitating membranes relaxed, and showed decerebrate rigidity. During the 90 minutes which immediately followed the operation such disturbances as manipulating the legs or moving the rectal thermometer caused only a short brief struggle accompanied by waving of the tail, protrusion of the claws, head movements from side to side and rises in arterial pressure and heart rate. Later these same disturbances had a more pronounced effect; they induced more vigorous and longer continued struggling and, in addition, facial movements suggestive of snarling, widening of the palpebral spaces, brisk retraction of the nictitating membranes and greater rises in arterial pressure (e.g., 166–204 mm. Hg) and heart rate (e.g., 206–256 beats per minute). Sweating did not occur nor did the respiratory rate rise above the normal. The arterial pressure remained above 140 mm. Hg and the quiet heart rate averaged around 210 beats per minute.

Experiment 50. Clean frontal transection at 9:53 a.m. It shaved the cranial borders of the superior colliculi and passed through the cranial part of the mamillary bodies. No spontaneous activity occurred at any time, but the toe pads were moist with sweat from 10:53 a.m. to 12:02 p.m. From 10:21 until the animal was killed at 2:30 p.m., moving the rectal thermometer or passively flexing a leg evoked a brief but strong fit of struggling accompanied by opening of the mouth, snarling, waving of the tail, widening of the eyes, brisk retraction of the nictitating membranes and increments in arterial pressure (e.g., 120–157 mm. Hg) and pulse rate (e.g., 230–252 per minute). When undisturbed the cat lay quietly with lids partially closed and eyes covered by relaxed nictitating membranes and showed decerebrate rigidity. The tail hairs were moderately erected throughout. The respiratory rate varied only between 22 and 27 per minute, the arterial pressure was at all times above 120 mm. Hg and the quiet heart rate gradually increased from 180, at the beginning, to 246 per minute at the end of the period of observation.

It is obvious that this sort of behavior falls short of the sham rage of such animals as cats 34 and 13 in being less vigorous and entirely dependent upon stimulation. At the same time it possesses a latitude which is absent from the reactions which can be similarly induced after more distal ablations. It is somewhat more expressive of an affective state. Particularly noticeable is the greater sympathetic activity; for example, the

ocular symptoms of cervical sympathetic discharge were readily induced and sweating occurred in two experiments (38 and 50) of this series. These changes were not seen when the transection passed more caudally. It is suggested that the basis of these differences is the presence, in the brains of these cats, of a small but definite fraction of that part of the diencephalon which has been shown to be requisite for the development of the sham rage.

Discussion. The work of a number of investigators, to which reference has been made, has established the fact that the decorticate cat or dog, in the chronic as well as in the acute condition, is capable of displaying a type of behavior which is commonly regarded as expressive of anger. From this it may be inferred that the nervous mechanisms for the expression of this emotion are subcortical. The results which have been presented in this paper support that view. They show that the remarkable activity which develops almost immediately after decortication in the cat and which is best described as a sham rage, is dependent upon the integrity of a definite part of the brain stem. Thus it regularly appeared after ablation of the hemispheres and all parts of the brain stem cranial to the middle of the diencephalon. But when, in addition, the caudal half of the thalamic region was removed the typical sham rage invariably failed to develop. By combining the results obtained from frontal transections with those which followed ablation of the dorsal part of the thalamus it becomes possible to state that the discharge of nervous impulses which evokes this extraordinary motor activity is conditioned by central mechanisms which lie within an area comprising the caudal half of the hypothalamus and the most ventral and most caudal fractions of the corresponding segment of the thalamus.

The consistency of the results obtained in these acute experiments is worthy of emphasis. In no case did the true quasi-emotional behavior occur when the area defined above was removed. In the great majority of those experiments in which it was left intact that behavior appeared in its full vigor. The consistency is further demonstrated by the observation that when a fraction of the active region remained the result was frequently a behavior transitional between that of the fully active animals and that exhibited by the quiet midbrain preparations.

Anyone who has ever tied an unruly cat to an animal board will agree that the sham rage shown in these experiments closely resembles the behavior of the infuriated normal animal. It has been pointed out that this is essentially different from the "pseudaffective reflexes" obtained in cats after section of the midbrain. The latter are less intense, less generalized, less easily invoked and far less expressive of an affective state. They appear to take their origin from a background of neural inactivity whereas

the phenomena shown in the decorticate or diencephalic preparation suggest hyperexcitability of the central mechanisms involved. A part of the central arrangements for emotional expression are of course situated below the level of the diencephalon. Certain elements of affective behavior may even be induced in the spinal cat, and still more in the bulbo-spinal or midbrain preparations, but the results of this investigation indicate that it is only when the diencephalic mechanisms are present that these elements can be readily welded together to form the rage reaction.

The question of the genesis of the decorticate rage cannot be answered with any assurance. Its spontaneity has been stressed, but it is realized that such a description is merely relative and serves to distinguish it from the less easily evoked "pseudaffective reflexes" of the decerebrate cat. Under the conditions of these acute experiments afferent impulses from the incised areas may be continually impinging upon the centers; and the restraint imposed upon the animal by tying it in the dorsal position is very probably a disturbing factor. The excessiveness of the activity as well as its easy elicitation gives it the appearance of a "release phenomenon." Thus it might be explained on the basis of the view first advanced by Hughlings Jackson (1884) and elaborated by Head (1921) to the effect that the cortex normally holds in check those activities of the lower and more archaic centers which would seriously interfere with its more discriminative reactions. The expression of emotional excitement is just this sort of activity and in the case of chronically decorticate animals release from cortical control must be the chief if not the sole factor in producing the tendency to react excessively; there the chronicity of the behavior precludes its being due to any sort of "irritation." But in acute experiments the latter may play a rôle. In these the widely opened cranium obviates the chance that the activity is, in any way, due to increased intracranial pressure. Nor could it be due to a cerebral anemia resulting from occlusion of the carotids, for it is recognized that in the cat the vertebral arteries are alone capable of maintaining an adequate cerebral blood flow.[1] Whatever the cause may be, it is one which activates a limited region in the brain stem.

The quasi-emotional behavior consists of both somatic and visceral

[1] This is implied in the demonstration by L. Hill (1896) that 60 per cent of cats will withstand ligation of both carotids and both vertebrals. Since no study seems to have been made of the results of occlusion of only both common carotids in cats, this procedure was carried out in four cats with aseptic precautions. These animals were watched carefully for six hours following the operation and were kept under observation for several days. In no case did any abnormal activity occur. One cat showed some mental dullness and slight ataxia during the first two days. The others appeared normal at all times after recovery from the ether.

activities and the latter are plainly due to a discharge of sympathetic impulses. Both appear to be the result of the activation of intimately related central mechanisms; they are not separable and together form an integrated reaction. The vigorous activity in skeletal muscle is difficult to account for on the basis of the present neurological status of the diencephalon, for, so far as is known, this part of the brain does not contain any definite somatic motor nucleus. Mesencephalic centers may in some way be involved, but this possibility is rendered unlikely by the fact that this division remained almost wholly intact in animals which did not exhibit the sham rage. On the other hand, there is good evidence that the sympathetic system possesses a definite diencephalic representation. Physiological evidence has been presented by Karplus and Kreidl. These investigators have found (1909, 1911) that in cats electrical stimulation of an hypothalamic point, lateral to the infundibulum, will produce maximal bilateral dilatation of the pupils, separation of the lids and retraction of the nictitating membranes. They showed that they were activating a true subcortical center (1910), that the ocular effects produced were mediated by the cervical sympathetic and that no other parts of the diencephalon except the hypothalamus yielded them (1909, 1910). They mentioned that the stimulation induced profuse sweating from all four feet and, in subsequent papers (1918, 1927), reported rises in arterial pressure. In their most recent investigation (1927) they found that the vascular effect persisted after removal of adrenals and hypophysis (which does not prove, as they seem to imply, that the stimulation does not affect the glands). Houssay and Molinelli (1925) have presented evidence that while weak electrical stimulation of the motor cortex, corona radiata, internal capsule and the thalamus has no influence on the adrenal medulla, the same current applied to the hypothalamus will cause a substantial secretion of adrenin. On the anatomical side the matter is less clear, but Greving (1925) has described nuclei in this region which he believes are related to the sympathetic system, and Dresel (1923) has offered some evidence that certain cell groups in the hypothalamus are connected with preganglionic neurones in the lateral horns of the thoracico-lumbar region of the cord.

Certain facts relating to the regulation of body temperature support the view that there is a diencephalic representation of the sympathetic. It is well known that erection of hair, ruffling of feathers, constriction of peripheral vessels and an increase in blood sugar occur when the homoiothermic animal is exposed to cold, and there is abundant evidence that medulliadrenal secretion is added to the blood under these circumstances (Cannon, Querido, Britton and Bright, 1927). These activities have an important place among the bodily changes which resist a lowering of body temperature. They are all due to a discharge of sympathetic impulses.

It is reasonable to suppose that this discharge has its source in the diencephalon, for it is this part of the brain which is essential for the maintenance of a constant body temperature. The labors of a large number of investigators (Isenschmid and Krehl, 1912; Isenschmid and Schnitzler, 1914; Rogers, 1919; Bazett and Penfield, 1922; Magnus, 1924) indicate that so long as this portion of the brain stem remains intact the processes of temperature control may proceed in a normal fashion.

Furthermore, exposure to cold and emotional excitement are attended by similar bodily changes. Restlessness, trembling and shivering, and especially those visceral activities that denote a widespread sympathetic discharge are prominent in both conditions. These reactions represent an effort on the part of the animal to cope with a critical situation and afford excellent examples of the emergency function of the sympathico-adrenal mechanism which has been emphasized by Cannon (1915, 1919). It now appears that in both instances they are dependent upon the activity of the diencephalon. This fact suggests that the diencephalic representation of the sympathetic presides over the emergency function of that system. The presumption is strong that it is not concerned in the simple reflex sympathetic discharges nor in the tonic discharge which occurs over certain fractions of the thoracico-lumbar outflow. Lower levels seem capable of subserving these functions; the pioneer work of Owsjannikow and Dittmar showed this to be true of the vasoconstrictor mechanism; Karplus and Kreidl (1918) admit that the integrity of their hypothalamic center is not necessary for the reflex activation of the cervical sympathetic; and Cannon and Rapport (1921) found that the reflex secretion of adrenin is not impaired by a midbrain section, but is wholly abolished by a transection just behind the inferior colliculi.

In view of these facts it is suggested that the present investigation has contributed evidence for the existence of a dominant central mechanism for the control of the sympathetic system, located in the diencephalon and responsible for the tendency of that system to discharge vigorously and as a whole under conditions of stress. In addition it is believed that the results obtained reveal the true significance of the various sympathetic effects which have been produced by stimulation of the hypothalamic region in narcotized animals. As isolated facts they possess little meaning; interpreted as the result of the activation of a dominant sympathetic mechanism they gain significance.

SUMMARY

An investigation has been made of the source, within the central nervous system, of a remarkable group of activities which follow decortication in the cat. This behavior simulates the expression of anger as seen in the normal cat and is best described as a sham rage.

The method used was that of ablation of varying portions of the brain stem after removal of the hemispheres. In acute experiments it was found that the typical sham rage regularly developed after removal of all parts of the brain cranial to the middle of the diencephalon, and after removal of the dorsal part of the thalamus. It invariably failed to appear after sections which separated the ventral and most caudal fractions of the lower half of the diencephalon from the midbrain. This leads to the conclusion that the expression of anger in the cat is dependent upon central mechanisms which are located in this part of the brain stem.

It is pointed out that the sham rage of the decorticate or diencephalic cat closely resembles the behavior of the infuriated normal animal and that it is far more readily elicited and is more expressive of an affective state than are the pseudaffective reflexes shown by decerebrate preparations.

The intense and widespread sympathetic discharge which is a prominent and invariable accompaniment of emotional excitement occurs during the sham rage. The relation of the diencephalon to the sympathetic system is discussed and the view is advanced that the diencephalic representation of the sympathetic consists of mechanisms which are responsible for the activation of that system under conditions of stress.

It is a pleasure to express my indebtedness to Dr. Walter B. Cannon who suggested this work and whose advice and encouragement carried it along.

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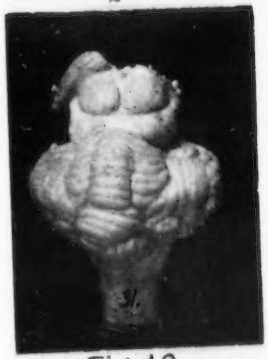

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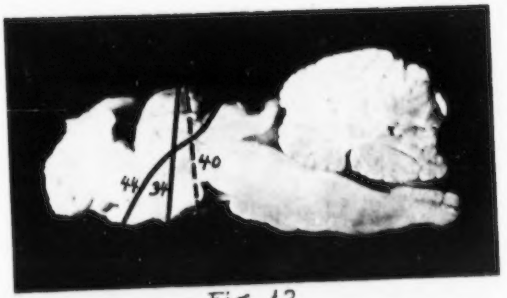

Fig 12

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EMPTYING OF THE GALL BLADDER IN PREGNANCY

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Recent investigations have indicated that the gall bladder of pregnant animals does not empty normally after the ingestion of fat (Mann and Higgins, 1927a, b, c). Since there is a high incidence of cholelithiasis in women who have been pregnant, this finding might have etiological significance, especially since the blood and bile of pregnant women have a high cholesterin content (Boyd, 1923; Fowweather and Collinson, 1927, and others). Thus bile stasis in pregnancy would favor the production of gall stones (Whitaker, 1927a). At least two cases have been reported, however, in which the gall bladder of a pregnant woman manifested normal emptying after feeding (Whitaker, 1927b, Bronner, 1928), and we have noted normal emptying in two pregnant dogs. It has been our opinion that the most important single factor in the emptying of the gall bladder is a state of physical well-being (Whitaker, 1926, 1927a). If this be true failure of the gall bladder to empty in pregnancy might result from associated gastro-intestinal or other disturbances. At least it would seem that if, in an animal as highly specialized as the cat, the gall bladder could consistently be made to empty in pregnancy, this condition would not in itself be an inhibiting factor.

In the series of five experiments with pregnant cats here presented the gall bladder in three almost completely emptied within the normal period (figs. 1, 3, 5). These animals were all in good condition, having fully recovered from the operation of filling the gall bladder with iodized oil. In the experiment represented by figure 4 the gall bladder showed normal though not complete emptying.

Very slight emptying was observed in figure 2. Though the cat was apparently normal it may not have fully recovered from the operation, only six hours before. Sometimes, however, the gall bladder unaccountably fails to empty in apparently normal animals. Unless taken into consideration this may be a source of experimental error.

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CONCLUSION

The gall bladder may not empty in pregnancy after the ingestion of fat; but neither may it empty thus in normal animals.

On the other hand the gall bladder in pregnant cats may completely empty after taking fat, provided the animal is in good condition.

Consequently pregnancy, in itself, exerts no inhibitory effect upon the emptying of the gall bladder.

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Fig. 1. *a*. Gall bladder of a fasting cat in advanced pregnancy, ten hours after being filled with iodized oil. Note the rounded, relaxed appearance of the fundus.

b. One hour after feeding 100 cc. of olive oil emulsion by stomach tube; elongation of the viscus due to muscular action; pronounced emptying.

c. Two and one-half hours after feeding; emptying continues.

d. Seven and one-half hours after feeding; gall bladder is reduced to a tubular organ with very small cubic content. Compare with *a*. Eight hours after feeding the gall bladder was found at necropsy to contain only two or three drops of fluid.

Fig. 2. *a*. Gall bladder of a fasting cat in advanced pregnancy, six hours after being filled with iodized oil.

b. One hour after feeding two egg yolks by tube; no emptying.

c. Two hours after feeding; slight activity of the viscus with expulsion of a few drops of oil.

d. Four hours after feeding, no further emptying. Contrast with figures 1 and 5.

Fig. 3. *a*. Resting gall bladder of pregnant cat, 24 hours after being filled with iodized oil.

b. One hour after feeding three egg yolks by tube.

c. Two hours after feeding; normal emptying.

d. Eighteen hours after feeding; gall bladder has refilled with bile. The duct and the fundus only are outlined by the few remaining drops of oil. Compare with *a*.

Fig. 4. *a*. Gall bladder of a fasting cat in advanced pregnancy, eight hours after being filled with iodized oil.

b. One hour after feeding two egg yolks by tube; pronounced emptying.

c. Two hours after feeding; emptying continues. Note the large amount of oil in the intestine. Compare with *a*.

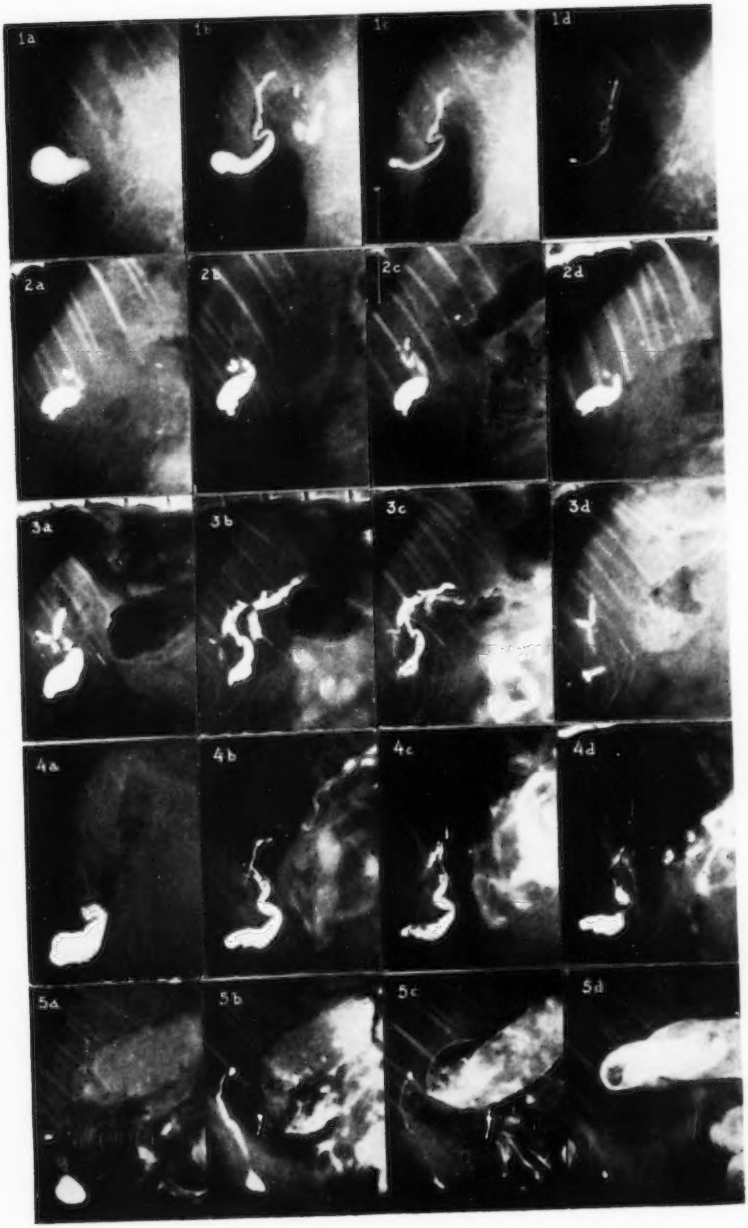
d. Four hours after feeding; emptying has ceased, gall bladder is relaxing. This is a normal reaction to feeding fat.

Fig. 5. *a*. Gall bladder of a fasting cat in early pregnancy. Twenty-four hours after being filled with iodized oil. For some reason there has been slight emptying; gall bladder appears relaxed now, however.

b. Fifteen minutes after feeding three egg yolks by tube; very rapid emptying.

c. One and one-half hours after feeding; emptying nearly complete.

d. Four hours after feeding; only a single drop of oil left in the gall bladder. Compare with *a*. Note oil in colon.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THE SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

V. Gastric Acidity[1]

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It was reported several years ago by Cohnheim and Kreglinger (1) that profuse sweating resulting from a fatiguing march caused a reduction of the gastric acidity. Believing that there was an insufficient amount of data reported by these investigators caused us to pursue the problem further, not by fatiguing exercise, but by sweating produced by heat.

The general system of sweating the subjects was the same as previously reported (2). The persons chosen for the experiments were chiefly university students and two working men on the campus, all of whom were apparently in good health.

The subjects were instructed not to partake of any food or fluids after retiring the night before until the time of their appearance at the laboratory the following morning. In some instances the subjects were used at noon in which cases they were instructed to eat a light breakfast at an early hour.

After coming to the laboratory they were given a test meal consisting of about 20 grams of dry toast without butter and 250 cc. of tea without cream or sugar. After the ingestion of this meal the subjects swallowed a modified Rehfus tube of the type that is used at the Hull Physiological Laboratory of the University of Chicago. A sample of the gastric contents was immediately aspirated that would yield 3 or 4 cc. of the filtered juice. Similar aspirations were made at ten-minute intervals to the end of the experiments. There were two or three samples obtained before entering the sweat cabinet and four to six samples while in the cabinet, all depending upon the case with which perspiration was produced. Two samples were usually obtained after retiring from the cabinet.

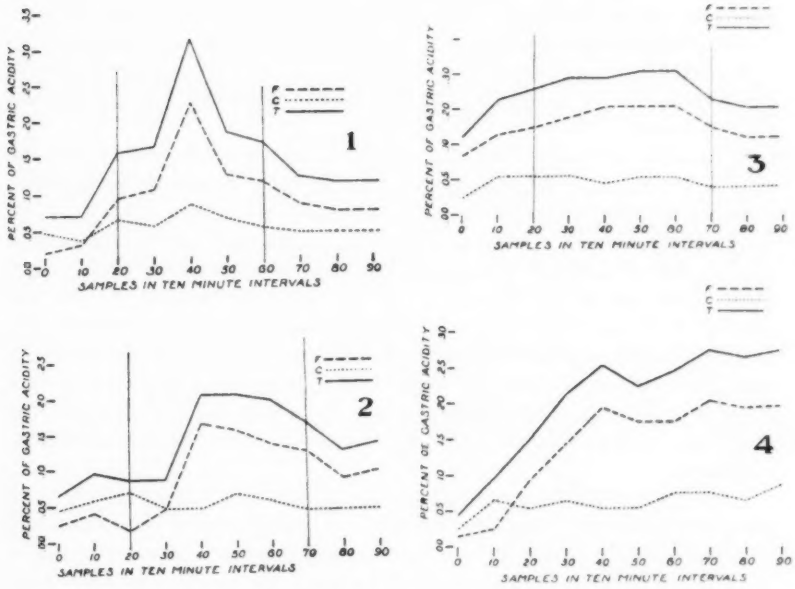
In some cases we removed the residual juice before partaking of the test

[1] The expenses for this research are partially defrayed by a grant from the American Medical Association Research fund.

meal which, of course, meant the swallowing of the tube before and after the meal. We believe, so far as our experiments are concerned, our results are equally significant without the removal of the residual juice, and the subject was saved the discomfort of swallowing the tube twice.

In our control experiments the details were exactly the same except that the sweating was omitted. This was done for the purpose of comparing the acidity curve in the two forms of experiments.

Immediately after aspiration 1 cc. of the filtered juice was diluted with 25 cc. of distilled water and titrated with one-fortieth normal sodium hy-



The horizontal bars seen in some of the figures indicate the time that the subject was in the cabinet. *T* stands for total, *F* for free, and *C* combined acidity.

droxide, using dimethyl-amino-azo benzene and phenolphthalein as indicators for obtaining of free and total acidity.

In our figures we have plotted the relative acidity on the ordinates and the ten minute intervals on the abscissa. The horizontal bars indicate the time that the subject was in the cabinet. Of course, in our control experiments these are omitted.

In all we performed 104 experiments of which 44 were controls. In our 60 sweat experiments we have endeavored to pick out the most typical cases which, for convenience, we have divided into three groups. The

first group is rather well typified in figure 1, where it may be noted that a rather precipitous dip in the acidity curve appears, as a rule, from 20 to 30 minutes after entering the cabinet, which corresponds pretty closely to the time when sweating is profuse. Group two represented here by figure 2, shows more of a sustained curve, yet, nevertheless, the fall usually occurs, as in group one, about 20 to 30 minutes after entering the cabinet. For group three we are presenting one graph represented in figure 3, however we have selected this, because it is farther removed from the types shown in the first two groups. In fact it presents a curve that much more simulates the control picture. About 70 per cent of our experiments belong to group one, about 20 per cent to group two, and 10 per cent to group three.

From the control experiments we are presenting figure 4 as being typical of over 90 per cent of the 44 control experiments. In comparing these with those produced in the sweating experiments, the sustained curve rather than the dip is seen.

It is not at all astonishing that we get a variety of fluctuations in the acid curve of the two lines of experiments, for there are several factors that must be kept in mind which might influence the same. Many times, where there is much nausea and retching, it will cause the swallowing of alkaline saliva and much mucus which would lower the acidity at the start. Some indications of the importance of this factor are noted in the observations of Kahn and Stokes (3) on gastric fistula patients in which they discovered a higher acidity in the stomach when the juice was removed directly from the fistula than when aspirated by a Rehfus tube through the esophagus. Luckhardt and Johnston (4) also have stressed the factor of the regurgitation of the alkaline intestinal juices which would have a neutralizing effect.

In our experiments the subjects, through much practice, became so accustomed to swallowing the tube that we doubt whether the nausea and retching would be much more than a negligible factor. If so, it would be only at the beginning, for there was a general rise in the acid curve soon after partaking of the meal. However, in making due allowances for all such possible sources of error, we believe the constancy, which the fall in the acidity after 20 to 30 minutes in the sweat cabinet and the more gradual decline in our control experiments indicate, leaves very little doubt that profuse sweating lowers the acidity of the gastric juice.

Presumably then the sweat glands in their requisition of chlorides of the body would reduce the hydrochloric acid of the gastric juice.

SUMMARY

1. Graphs are presented which show that in heat sweating there is a more or less sudden decline in the gastric acidity which is in accord with the report of Cohnheim and Kreglinger on sweat produced by exercise.

2. Graphs in the control experiments, where sweating was omitted, show a more sustained acidity until the end of digestion.

We wish to convey our thanks to Doctor Luckhardt for sending us a sample of the Rehfus tube that is used in the University of Chicago laboratory; also we are greatly indebted to Doctor Hektoen and the members of his committee for the financial aid granted to us from the American Medical Association Research Fund, for not only this part of our problem but for other parts to be reported.

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THE EFFECT OF CARBON ARC RADIATION ON CIRCULATION IN THE DOG[1]

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There is a general view that irradiation, particularly with the C arc, produces a greater or less depression of arterial blood pressure, although a rise, during or soon after irradiation, has been reported. The factors held responsible are diverse. The literature has recently been reviewed by Laurens (1928).

Hasselbalch (1905) and Hasselbalch and Jacobaus (1907) described decreases in blood pressure averaging between 8.5 and 10 per cent and lasting for several months following massive irradiation with a large C arc lamp given at such intervals as to maintain lasting hyperemia with the avoidance of pigmentation. The altered blood distribution was believed to be the cause of the lowered pressure. Reed (1923, 1925, 1925b) obtained marked decreases in pressure and heart rate in dogs anesthetized with ether or chloretone when their atropinized eyes were illuminated with strong mazda lamps or a C arc. He ascribed the results to effects on the blood, since he obtained similar results when the mucosa of the mouth and pharynx, or the blood as it flowed through a quartz tube interposed in the carotid or femoral artery, was irradiated. Rothman (1923) is convinced that the depression in blood pressure is not the result of cutaneous hyperemia, because it is possible to obtain a drop in pressure without any cutaneous hyperemia and no drop in pressure when the hyperemia is marked. Further, that it is not dependent upon the breathing of nitrous oxide as claimed by Kestner (1921, 1922), Kimmerle (1921, 1922), Peemöller (1923) and Knipping (1923). The depression sets in gradually and lasts for several days and Rothman believes that its primary cause is sympathetic hypotonia, a view to which Petersen and Öttingen (1927) subscribe.

The effects on pulse rate, body temperature, etc., vary according to different observers, due in great measure to varying experimental or clinical conditions. Hasselbalch and Lindhard (1911) assert that ultra violet has no marked effect on pulse frequency, perhaps slightly retarding it, while Durig, von Schrötter and Zuntz (1912) describe an increase in pulse frequency as a result of intense illumination. Mayer (1926) reports that

[1] Aided by a grant from the David Trautman Schwartz Research Fund.

ultra violet rays alone seldom affect the pulse rate, but that intense sunlight may cause tachycardia. The heat is more likely to be the factor here. Mason and Mason (1927) found, along with a decreased basal metabolism, a lowered pulse rate as a result of irradiating patients with a quartz Hg lamp. Sonne (1921) found that the luminous rays as emitted by a C arc heated a very essential portion of the aggregate blood volume to a temperature exceeding the highest clinical fever noted without causing the body temperature to rise, while Rosselet (1922) states that the temperature of the skin may exceed 104°F. during insolation and that direct warming of the blood is concerned in the rise of body temperature which takes place during a sun bath.

It is significant that diastolic pressure has been neglected and that attention has been concentrated on the immediate effects of exposure. On this account as well as because of the contradictory nature of many of the reports it was regarded of interest to determine the temporary or long lasting changes in systolic and diastolic pressures, pulse rate and body temperature of normal dogs under standard conditions following exposure to moderate or massive doses of C arc radiation.

The desirability of a simple and accurate method of determining blood pressure, systolic as well as diastolic, without anesthesia, operative procedure or other disturbance, in laboratory animals, especially in the dog, has long been recognized (see Allen (1923) whose application of the auscultatory method to the dog we have used with marked success). We employ a Tycos sphygmomanometer, a child's cuff and a small Pilling phonendoscope with metal rings soldered to each side of the bell to which tapes are attached.

Adult dogs kept in the laboratory on a standard maintenance diet are trained to lie quietly on their sides without being tied. The lower part of the thigh, previously shaved, is palpated for the course of the femoral artery, the phonendoscope bell is applied to the artery and tied in place, and the cuff wound around the leg so that its lower portion covers the bell. A small band, made from an automobile tire tube cut to the width of the cuff, is placed over it and keeps it in place when inflated. The sounds obtained with this method are not as loud but are practically as sharp and plain as in human subjects and with a little practice readings can be taken as easily and as quickly as in man. The animals quickly become accustomed to the routine and show little concern in the procedure. Occasional refractory animals, or those whose sounds were indistinct, were discarded at the outset.

The observations were begun in the morning at approximately the same hour each day and blood pressure, pulse rate and temperature readings were taken in triplicate after the animal had been on the table for at least a half-hour. Preparatory to irradiation, readings were made on several

days at 15-minute intervals for a period equal to or longer than that of the proposed exposure as well as in the afternoon. This was done to insure a correct interpretation of the changes; that is, as to whether they were due to the specific action of radiation or to the effects of rest, training, etc., and to obtain information as to normal diurnal variation. During irradiation periods readings were made for one-half hour before the lamp was turned on, and continued at 15-minute intervals for one-half to one hour after the irradiation was over and resumed again in the afternoon. This enabled us to observe not only the progressive changes from the initial normal values but also the effects of each individual exposure as well. The animals were always followed after the irradiation had been discontinued until the values approximated normal. The eyes of the animals were protected from the direct radiation. The "Majestic Arc" (Solarite, 25–28 A., 40–50 V) and the "Pan-Ray-Arc" (25–28 A., 50–60 V) served as the radiation sources. The total energy of these lamps and its spectral distribution have already been specified (Mayerson, Gunther and Laurens, 1926; Laurens and Mayerson, 1927).

Since readings were made daily at 15-minute intervals before, during and after irradiation, a detailed presentation of all the data accumulated is impossible, and the averages of readings taken during the various periods and abbreviated protocols are used to present the data.

While there are fluctuations throughout the day in blood pressure, pulse rate and body temperature, the averages of these values for the same time on consecutive days show very constant levels. There is a slight decrease in pulse rate at the end of the morning, due to the rest and quiet of the animal while on the table, which is recovered from by early afternoon. The averages of 20 weeks of daily readings on dogs placed on the table for an hour and not irradiated show but slight variation.

A total of 14 experiments on 9 dogs have been performed. In the experiments with the Majestic Arc the animals were irradiated daily on the back or the abdomen at 40 cm. from the lamp for periods ranging from three-quarters of an hour to two hours (40 to 110 g. cal. per sq. cm.). The responses are of two sorts, *1*, temporary changes with return to normal levels within 24 hours; and *2*, effects which persist after the irradiation is discontinued.

The first exposure to the Majestic Arc produces very little effect except when the abdomen is irradiated, in which case there is an average fall of about 10 mm. (1 per cent) in systolic pressure accompanied by a rise of about 15 mm. (1.5 per cent) in diastolic, the values returning to normal within 5 hours after the irradiation is over. Definite changes in the same direction are evident in all cases at the third exposure, particularly in abdominally irradiated animals, as shown in table 1, embodying the percentage changes in 4 experiments for each type of exposure. The systolic

pressure begins to fall within 15 minutes, and continues to do so during the irradiation, reaching its lowest point soon after the end of the exposure (average drop is 4.9 per cent). The diastolic pressure shows a varied response. In the four experiments where the back of the animal was irradiated, there is an average increase of 18 per cent, while the abdominally irradiated animals show an average decrease of similar magnitude. In two experiments with abdominal irradiation the diastolic pressure drops 30 to 21 per cent respectively at the end of the irradiation, returning slowly to normal in 6 hours. The heart rate shows a more marked rise on abdominal exposure, and the pulse is fuller and stronger and is characterized by the disappearance of irregularities.

In the experiments on dorsal irradiation, the circulation rate (pulse rate times pulse pressure) shows an average decrease of 22 per cent at the end of the third exposure as a result of the decrease in pulse pressure

TABLE 1

Average per cent changes following third exposure

	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE (P.P. × P.R.)	TEMPERATURE
						°C.
Back (4 animals).....	−2.5	+18	−26	+4	−22	+1.5
Abdomen (4 animals)...	−7.3	−18	+14	+10	+23	+1.6

(decreased systolic and increased diastolic), while abdominal irradiation, followed by an increase in pulse rate as well as in pulse pressure, leads to increased circulation rate. The increased diastolic pressure in the former suggests increased tone of the smaller blood vessels as a compensatory reaction to the fall in systolic pressure. Following the more effective abdominal exposures the pulse pressure is high (lowered systolic and diastolic) indicating loss of tone and dilatation of the small vessels. The marked increase in the pulse rate under these conditions may again be regarded as a compensatory reaction, and the circulation rate rises above the normal value during the irradiation, remaining so for an average of two hours thereafter. Body temperature increases during the irradiation, the greatest change being 1°C., with recovery at most within 24 hours after the irradiation.

Successive exposures intensify the changes, the maximum effects being obtained at the fifth, after which there is no greater effect. Figure 1 illustrates a typical case. The pulse pressure drop in this instance is smaller than the average and the circulation rate remains more constant, being slightly high at the end of the exposure and in the afternoon. Table 2 shows the averages of the values for the fifth irradiation in 5 experiments.

In all but one (A 2, abdominal) the decrease in the systolic is accompanied by a similar fall in the diastolic at the end of the irradiation and by a decrease in pulse pressure which is recovered from by the next morning. The pulse rate usually increases during the exposure and the body temperature rises slightly (maximum of 1.3°C.), both returning to normal by the next morning. The increase in pulse rate is similar to that found in the third exposure and probably represents a compensation for the decrease in pulse pressure in maintaining a constant circulation rate. The decrease in pulse

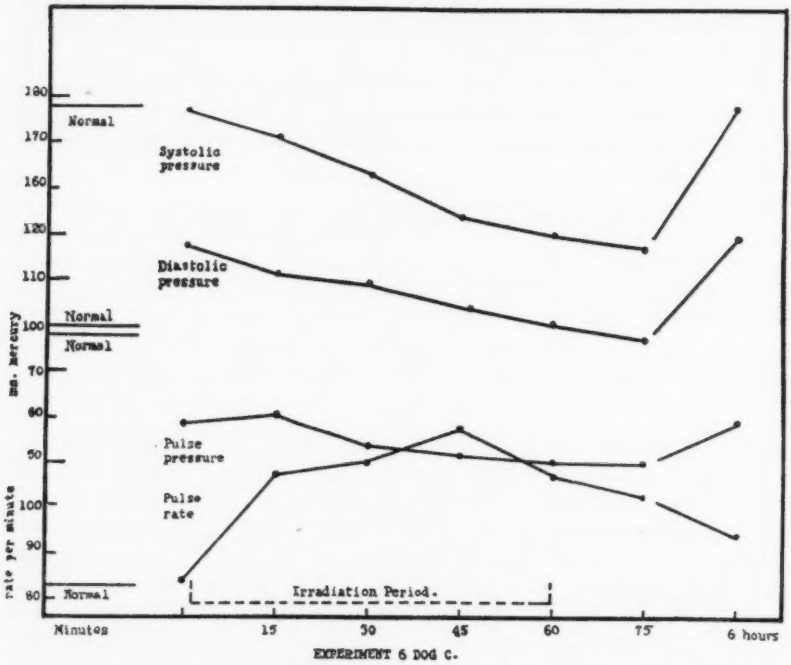

Fig. 1. Fifth abdominal irradiation with Majestic Arc for 1 hour at 40 cm. (55 g. cal. per sq. cm.).

pressure, however, is usually proportionately greater than the increase in pulse rate and there is an average decrease of 20 per cent in the circulation rate during and after the irradiation. On the other hand, the value is usually above normal by the next morning, an indication of over-compensation on the part of the heart and the circulatory system.

Only exceptionally do repeated exposures after the fifth give any greater or longer lasting effects even when the daily exposures are repeated for a considerable length of time. Dog D is one of these exceptions. On the

8th exposure, the systolic pressure fell from 184 to 142 mm., fluctuated during the afternoon and was at 146 mm. the next morning. On the 10th day the pressure was at 155 mm., the irradiation lowering it again to 140 mm. The diastolic pressure decreased 18 mm. (17 per cent) but was back to normal soon after the exposures and the pulse rate and body temperature also did not show any lasting changes.

In this case the systolic pressure remained low for about 72 hours, indicating the possibility of obtaining a longer lasting depression under more optimal conditions. All of the animals used up to this time were more or less pigmented, thus reducing the action of the radiation by absorption, as indicated by the earlier and more marked changes following abdominal

TABLE 2

Effects following fifth daily irradiation

	SYSTOLIC PRESSURE			DIASTOLIC PRESSURE			PULSE PRESSURE			PULSE RATE			CIRCULATION RATE		
	Before	After	24 hours after	Before	After	24 hours after	Before	After	24 hours after	Before	After	24 hours after	Before	After	24 hours after
Dog A (1)	148	120	148	80	68	86	68	52	62	76	80	100	5,168	4,160	6,200
Dog A (2)	144	124	154	72	93	80	72	31	74	92	100	86	6,624	3,100	6,364
Dog C	168	140	184	121	92	108	47	48	76	88	112	80	4,136	5,376	6,080
Dog D	182	147	174	123	104	115	59	43	59	104	100	116	6,136	4,300	6,844
Dog E	161	142	169	81	75	77	80	67	92	80	84	80	6,400	5,628	7,360
Average	160	134	166	95	86	93	65	48	72	88	95	92	5,692	4,512	6,569

Average per cent decrease: systolic = 16, diastolic = 10, pulse pressure = 26; pulse rate increase = 7.5; circulation rate decrease = 20.

Dogs A (1), A(2), C: abdomen exposed. Dogs D, E: back exposed.

irradiation. Accordingly a white, short-haired animal was irradiated daily 6 times on the back and 13 times on the abdomen. The first exposure caused a slight decrease in systolic and a slight rise in the diastolic pressure, and subsequent exposures were followed by changes similar to those described. On the twelfth day of irradiation, however, the systolic pressure dropped from 160 to 130 mm., and decreased still further to 108 following irradiation on the next day (a maximum depression of 36 per cent). It then rose to 130 mm. by the next morning where it remained until the 19th day, when the irradiation was discontinued. Recovery to approximately normal levels occurred on the 22nd day, three days after the last exposure and 10 days after the marked drop. The diastolic pressure rose during the twelfth irradiation but decreased to about 20 per cent below normal by the next morning where it remained for the next two days. It rose again

during the next exposure but came back to the low level the next morning. Since both pulse pressure and pulse rate were low, the circulation rate was below the original value, an average of 30 per cent.

Judging from the marked effects obtained in the above experiment it seemed probable that more intensive irradiation of white, short-haired dogs would accentuate the changes. A dog (Z) was therefore irradiated abdominally with the Pan-Ray-Arc three times with a dosage of 45 g. cal. per sq. cm.; 5 times with a dosage of 50 g. cal. per sq. cm.; once with 70 g. cal. per sq. cm.; and 4 times with a dosage of 166 g. cal. per sq. cm. for a total of 13 daily exposures. The changes were in the same direction and, with the exception of diastolic pressure, of greater magnitude than those previously observed, and indicate that the effects are to some extent proportional to the intensity of the radiation. The values for these various exposures averaged on a percentage basis are given in table 3.

No lasting depression, however, was observed even after the strong individual exposures. It seemed, nevertheless, that if such irradiation were

TABLE 3

Average per cent changes with progressively strong abdominal exposures

DOSE	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE (P.P. × P.R.)	TEMPERATURE
						°C.
45 g. cal. per sq. cm...	−13	−16	−14	+5	−9	+3.0
50 g. cal. per sq. cm...	−17	−9	−23	+21	−13	+3.5
166 g. cal. per sq. cm...	−19	−10	−25	+40	−18	+7.0

continued there might be a resultant prolonged depressor action. But since such massive doses daily administered for any considerable period produce pathologic burns and blisters it was decided to irradiate at intervals but often enough to maintain hyperemia. Two dogs (X and W) were accordingly irradiated with the Pan-Ray-Arc, dog X with a dosage of 83 g. cal. per sq. cm. at intervals of 2 and 3 days respectively, followed in 6 days by an exposure to 124 g. cal. per sq. cm., and dog W with 4 exposures of 83 g. cal. per sq. cm. at the same intervals.

The first irradiation was accompanied by a decrease in systolic pressure of 14 and 13 per cent respectively, a diastolic drop of 17 per cent in dog X, and a rise of 6 per cent in dog W. All values were back to their original levels in 4 hours except the diastolic pressure in dog X which remained low for over 24 hours. The second exposure gave similar changes with recovery in the afternoon of the same day. After the third exposure, 3 days later, there was a decrease of 30 and 25 per cent respectively in systolic and diastolic pressures in dog X and 22 and 13 per cent respectively in dog W.

The pressures remained low with gradual recovery in 6 days. The fourth irradiation (6 days after the third) was again followed by a fall in the systolic of 24 per cent in dog X with little change in the diastolic. In dog W the systolic and diastolic pressures were decreased 11 and 14 per cent respectively. Both, however, were low the next day and remained so until the seventh day after the last irradiation when they began to rise and reached original values on the twentieth day (see table 4).

The pulse rate usually increased during the irradiation and decreased after it, the changes being in the reverse direction to those in the pulse pressure. In the second experiment (dog W) the rate was only 20 per cent normal 15 days after the last exposure. The circulation rate decreased after each irradiation, maximum changes of 59 and 37 per cent respectively following the last exposure, with the levels remaining 14 and 20 per cent below the original through the post-irradiation period. The body tempera-

TABLE 4

Effects following strong abdominal irradiation

	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE (P.P. × P.R.)	TEMPERATURE
						°C.
Normal	171	70	101	85	8,585	38.1
Immediately after last irradiation	122	79	43	82	3,526	38.0
1st day after	124	47	77	118	9,086	39.4
5th day after	123	61	62	88	5,456	38.4
10th day after	151	54	97	91	8,827	38.7
15th day after	152	68	84	84	7,056	38.4
20th day after	168	67	101	73	7,373	38.1

ture showed a maximum increase of 2°C. which was gradually recovered from except after the last irradiation when for 2 days the temperature remained 1° and 0.5° respectively above normal.

Our observations on a total of 166 separate exposures unquestionably confirm those of other investigators who find that C arc radiation depresses systolic pressure. That this is due to the direct action of the radiation and not to the inspiration of combustion products of the arc was shown by the lack of changes in control dogs placed behind a screen at the same time that the dog was being irradiated and at the same distance from the arc. The decrease in pressure begins almost immediately and usually reaches its maximum at the end of the exposure or soon after, its extent being dependent on the intensity of the radiation. Abdominal irradiation of a dog for 1 hour at 40 cm. (55 g. cal. per sq. cm. per min.) decreased systolic pressure to the same extent as did a two hour dorsal exposure of the same animal at the same distance from the lamp. That the depression is roughly

proportional to the dosage is also illustrated by the results of experiment 12 (dog Z, table 3) and more strikingly by the following. Dog F, which had been exposed daily to 22 hourly irradiations (55 g. cal. per sq. cm.) of the Majestic Arc with no effect, was irradiated with the stronger Pan-Ray-Arc for one and a half hours at the same distance (40 cm.) (562 g. cal. per sq. cm.). In 10 minutes the systolic pressure decreased from a normal pre-irradiation value of 150 mm. to 110 mm. In an hour it had fallen to 58 mm., the animal at this time exhibiting convulsions and symptoms of shock. At the end of an hour and a half the light was turned off, the pressure having dropped to 38 mm. The animal died 15 minutes later.

The changes in diastolic pressure, pulse pressure and pulse rate indicate that the first response of the circulatory system to radiation is an increase in the tone of the capillaries and smaller blood vessels accompanied by an increased pulse rate, which, when the irradiations are continued or intensified, gives way to a peripheral dilatation, loss of tone. In order to maintain a proper circulation rate the pulse rate further increases, the rise being proportional to the amount of diastolic depression.

Hasselbalch (1905) believed that it is possible by irradiation to obtain a greater or less drop in systolic pressure owing to partial, or temporarily complete, depression of cutaneous vascular tone. But even if treatment is continued, this can not go beyond a certain lower level presumably fixed by the more or less unchangeable capillary resistance and succeeding erythema-producing doses can not give any further drop. In the experiments where we obtained a maintained depression of systolic pressure, successive exposure prolonged but did not accentuate it.

Lewis (1926) and Lewis and Zotterman (1926) point out that the reaction of the cutaneous vessels to ultra violet radiation is similar to that of tissue injury as in freezing and burning, consisting in local dilatation, reflex dilatation and increased permeability. The underlying cause of these reactions is the liberation of substances having histamine-like action which diffuse out into the surrounding skin and are conducted away by lymphatic channels. The vessels become dilated because they lose their contractile power.

Our experiments show that in certain normal dogs following massive doses of C arc radiation repeated at intervals it is possible to produce a sustained depression of arterial pressure. Since the normal animal is a relatively stable mechanism as contrasted with the abnormal, the method should be especially adapted to the treatment of hypertension.

SUMMARY

Moderate C arc irradiation of the abdominal area of dogs results in a decrease of 5 to 20 per cent in systolic pressure, after either the first or

second exposure, persisting for about 5 hours and followed by a gradual return to normal. The diastolic pressure, in some cases, shows a proportional decrease; in others it rises during irradiation and returns to normal soon after. When the back of the animal is exposed similar changes are evident after the third or fourth dose. The effects in all cases usually reach a maximum on the fifth exposure and re-occur with each subsequent irradiation. With few exceptions all values tend toward normal levels within 24 hours after each exposure. The pulse rate usually increases during irradiation but this effect is not consistent. The circulation rate usually decreases during the irradiation, recovering in 24 hours to values frequently greater than the original. Body temperature shows little variation, slight rises occurring during irradiation disappearing soon after the exposure is over. If the daily dosage is progressively increased, the magnitude of the changes is roughly proportional to the intensity of the radiation.

Long lasting depression of the blood pressure was obtained in three experiments with white, short-haired animals. In one experiment, after the twelfth daily irradiation the dog showed a decrease of about 20 per cent and 15 per cent in systolic and diastolic pressures respectively, the values remaining at this level through 5 subsequent exposures and for three days thereafter before returning to normal. The most marked effects were obtained in massive irradiation (83 to 124 g. cal. per sq. cm.) of two animals at intervals of 2, 3 and 6 days respectively where the depression of the systolic pressure was maintained for 15 and 20 days respectively with values 12 and 22 per cent below normal, and the diastolic for 15 and 10 days with values 12 and 21 per cent below normal.

Control dogs placed behind a screen at the same distance from the lamp while the experimental animal was being irradiated showed no changes in blood pressure, pulse rate or body temperature.

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THE EFFECT OF CHANGES OF pH ON THE CARDIAC ACTION CURRENT[1]

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It has been known for some time that changes in pH do alter the form of the action current of the heart, as recorded on the string galvanometer. Andrus and Carter (1924) give a historical sketch of the subject, quoting the chief contributions of the various authors. In a word, previous studies have shown that an acid solution reduces the force of the heart beat and impairs the a-v conduction (Daly and Clark, 1920); that an alkaline solution increases the heart rate and decreases the conduction time (Mines, 1913; Andrus and Carter, 1924); that the effect is still more profound in the absence of oxygen (Drury and Andrus, 1924); and finally that there is an abrupt change in the duration of the refractory period at pH 7.4, the time increasing to the acid side and decreasing to the alkaline side (Carter and Dieuaide, 1926). Mines (1913) in a study dealing with the interpretation of the T-wave, notes that the direction of this wave can be reversed between the limits CH $10^{-8.9}$ and CH $10^{-6.6}$, giving pen sketches to show the effect.

Amberson and Klein (1927) have found the electromotive forces developed across the frog's skin, when it separates solutions of different concentrations (the so-called "concentration effect") are greatly influenced by changes in pH. Above a certain pH the more dilute solution is electropositive, below this point the more dilute solution becomes electro-negative. A reversal of the usual direction of the electromotive forces is thus achieved by making the solutions more acid. Somewhat similar phenomena have been observed in the electromotive forces developed in the frog's gastrocnemius when injured. The reversals in these tissues occur at a pH level much below 7.4 and the chronaxie values of Fredericq (1925) and of Lapique and Larrier (1926) make a low reversal point not unlikely even in heart tissue. These observations suggested that action currents might possibly show similar modifications, or even reversals in direction, when pH

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is lowered. The electrical variations in spontaneously beating hearts have, therefore, been investigated from this point of view. It is not our purpose in this communication to offer an explanation of the theory involved, but rather to indicate our procedure and present actual records for consideration.

METHODS. Turtle hearts were perfused with the perfusion apparatus described by Dawson (1925). With this apparatus the fluid can be changed by closing one stopcock and opening another, or the bottles can be removed and others substituted. Turtles were stunned by a blow on the head, the hearts were quickly removed and attached to the perfusion apparatus. In order to keep the perfusion pressure constant, similar amounts of fluid (30 to 50 cc.) were placed in the bottle and allowed to run through. When the last few drops were leaving the cannula, the electrocardiographic tracing was taken. As a result, in each instance, the record was taken with the perfusion pressure at zero. A side tube allowed refilling of the cannula without the introduction of air bubbles, hence the experiment was completed with no interruption of the perfusion. The hearts were perfused at room temperature (19 to 20°C.) and in any given experiment, the maximum temperature variation, recorded inside the perfusion cannula, was not more than 1°C. The experiments were all made during the month of June, on fasting turtles.

In one experiment reported, oxygen was not used. In all other experiments oxygen was bubbled through the perfusion fluid at such a rate that the bubbles could not be counted.

The electrodes were non-polarizable, consisting of strips of silver coated with silver chloride and embedded in saline agar in small glass tubes. Actual contact was made by a cotton wick which was attached to the heart by a very superficial stitch. A slight slack in the wick allowed for the cardiac movements without causing any shift in the position of the electrodes which were securely fixed in boot electrode holders and clamped to a ring stand. In some cases a thread was attached to the apex of the heart and then connected with a Harvard muscle lever which was suspended before the camera so that a record of the mechanical contraction could also be photographed.

The hearts were sometimes perfused by way of the aorta and at other times the cannula was placed in one of the great veins leading to the right side of the auricle. In no instance had the heart ceased to beat at the end of an experiment; often the hearts would beat for two or three hours more if allowed to lie in Ringer's solution. Regardless whether the perfusion was from the arterial or the venous side, and regardless of the position of the electrodes, the results were in the same direction in every experiment.

Various perfusion fluids were used. One experiment reported was made with Ringer's solution (NaCl 0.7 per cent, KCl 0.03 per cent, $CaCl_2$ 0.025

per cent, $NaHCO_3$ 0.01 per cent), but for the other experiments, the formula of Sørenson, quoted by Mines (1913) was used. This solution is recommended, because of the borate-acetate buffer, where wide variations in pH are to be used for it obviates any change in ionic content by precipitation of the carbonates. In some instances the solutions were made acid by HCl and alkaline by NaOH while in others the acid effect was obtained by bubbling CO_2, from a Kipp generator, through the perfusion fluid. The total range of pH used was from 3.0 to 9.6. The pH of the solutions was tested colorimetrically by comparison with a new set of Lamotte standards. The dye solutions were freshly made. All solutions were kept in Pyrex flasks. During an experiment the return fluid was collected and in many experiments again tested, thus giving an idea of the direction of the pH change which occurred while the fluid was in contact with the tissues.

The tracings were taken, with standard technique, on a Boulitte electrocardiograph. At times the potential differences were so great that it was necessary to have the string tension so that 1 mv. = 0.5 cm. in order to record the full deflection on a 6 cm. paper. In a few instances, after treatment with acid a standard string was used. The resistances were always low, generally about 1000 ohms.

Results. In general the results of the earlier workers are confirmed, that is, the heart rate and the conduction rate are found to be more rapid in alkaline solutions, and the T wave is easily reversed by changing from an acid to an alkaline solution. These effects were more easily obtained and were even more profound in the absence of oxygen. No study has been made of the refractory period. In addition to these observations, we find that the main deflection (R wave) can also be reversed; there is no difficulty in changing the form of the whole complex. Perhaps these facts are more easily brought out by referring to the actual experiments.

Figure 1 shows six tracings from experiment 1, where Ringer's solution without oxygen added was used. 1a is the control record taken just before starting the perfusion, when an a-v block was present. Such a preparation would certainly be somewhat acid because of the accumulation of metabolites, especially because of accumulating lactic acid. Positive P, R and T waves, with an a-v block are recorded. After perfusing with an alkaline solution for nine minutes 1b was taken, which shows a negative T with a final positive deflection. Records 1c and 1d, obtained after perfusing with a solution at pH 3.0 for intervals of a minute, are not easily interpreted. They serve to show how utterly changed the electrocardiograph becomes in an acid solution and also from what degree of abnormality recovery can occur. Figure 1e is that obtained after returning to an alkaline solution, pH 7.8, for one minute. Three minutes later 1f, which is almost identical with 1b was recorded. In a space of six minutes, then, these changes can

be produced and reversed. Aside from the changes in the form of the wave, the slowing in the acid solution and the return to the faster rate in the alkaline solution, are well seen.

Figures 2 and 3 show that these changes are not due to the particular placement of the electrodes nor to the arrangement of the musculature. After twice alternating acid and alkaline solutions in experiment 1, the auricles were cut away, considerable tissue being sacrificed at the base to insure only ventricular tissue being left. The contacts were then placed at the right lateral base and at the tip of the apex. Figure 2a shows the form of the waves when the ventricle was bathed in the solution at pH 7.8, there are present a Q, a positive R, a deep S, and a final positive deflection

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Fig. 1. Full description in text. In all records time is in $\frac{1}{5}$ and $\frac{1}{25}$ second. In the electrical records an upstroke indicates primary negativity.

1a. Excised turtle heart. No perfusion. 2:25 p.m. String tension throughout the experiment 1 mv. = 0.5 cm.

1b. Perfused with Ringer's solution, no oxygen supplied. pH 8.0. 2:34 p.m. Note the changes in the downstroke of R, also in S and T.

1c. Perfused with Ringer's solution at pH 3.0. 2:35 p.m. Note the absence of any recognizable QRS group and also the slowing of rate and conduction.

1d. Perfused with Ringer's solution at pH 3.0. 2:36 p.m. Note the smoothing of the curve and the decreased amplitude.

1e. Perfused with Ringer's solution at pH 8.0. 2:37 p.m. Immediately upon changing to the alkaline fluid. Note the reappearance of a positive QRS group.

1f. Perfused with Ringer's solution at pH 8.0. 2:40 p.m. Compare this recovery with the control record 1b.

Fig. 2. Full description in text. Excised turtle heart.

2a. Isolated ventricle, beating spontaneously. Bathed with Ringer's solution pH 8.0.

2b. Same preparation bathed with fluid at pH 3.0. Again note the decrease in amplitude and the smoothing of the curve which is definitely negative and monophasic.

Fig. 3. Full description in text. Excised turtle heart.

3a. Isolated auricle bathed with fluid at pH 8.4.

3b. Same preparation 3 minutes after change to fluid at pH 3.0. Note the depression of the main deflection and also that the whole complex is recorded to the negative side of the isoelectric level.

Fig. 4. Full description in text. In this and subsequent records the lower tracing records mechanical contraction. Systole is indicated by a downstroke.

4a. Turtle heart perfused with Ringer's solution, pH 7.8. 10:55 a.m.

4b. Same preparation perfused with Mines' solution, pH 8.0. 11:00 a.m.

4c. Same preparation perfused with Mines' solution, pH 7.3. 11:12 a.m. The return fluid was at pH 7.5 Note the changes in the P and R waves.

4d. Same preparation perfused with Mines' solution at pH 6.2. Note the complete reversal of the main deflection.

Fig. 5. Full description in text. Turtle heart with perfusion fluid at pH 7.8. Note that an A-V block is present and that a complex group of waves corresponding to the usual P, QRS and T waves is present for the auricle. This group of waves is included within the white circle. The lettered waves are ventricular.

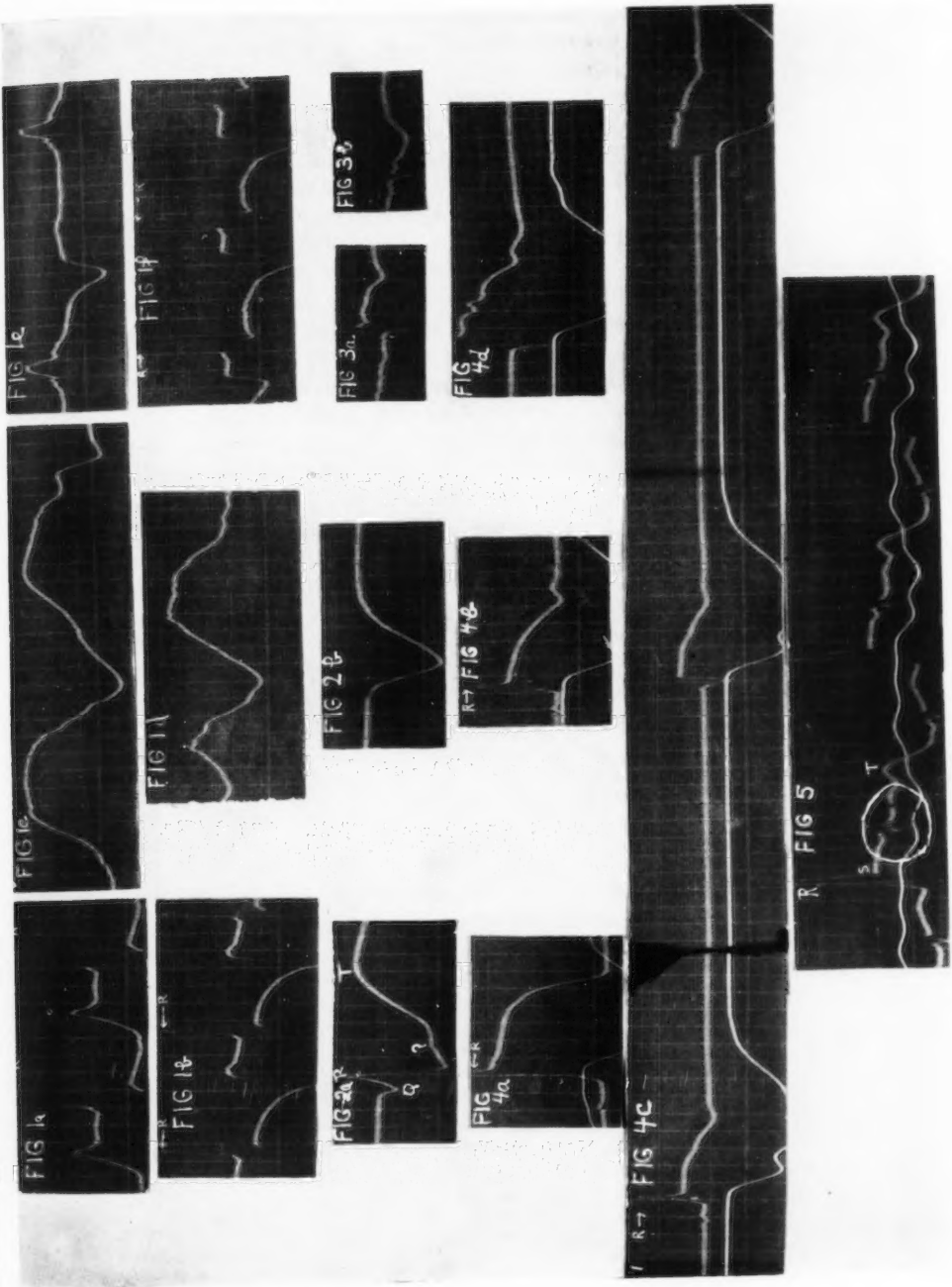
FIG 1a
FIG 1b
FIG 1c
FIG 1d
FIG 2a
FIG 2b
FIG 3a
FIG 3b
FIG 4a
FIG 4b
FIG 4c
FIG 4d
FIG 5
R
T
S

of the T. Figure 2b shows the same preparation after being bathed in an acid solution, pH 3.0, there remains a negative monophasic wave. A similar result was obtained from auricular tissue. In this preparation the perfusion was from the veins and the fluid continued to pour over the auricle after the ventricle had been cut away. Figure 3 demonstrates the changes in the auricle; in 3a the main deflection is positive while in 3b the whole complex is recorded to the negative side of the isoelectric level.

In an experiment where oxygen was supplied and where the mechanical beat was recorded, the same reversal occurred. The perfusion fluid was that recommended by Mines and several different solutions differing by about one tenth of a pH were made up so that the heart could be rapidly perfused over a range from 8.0 to 3.0. Figure 4a shows the control record where the fluid was Ringer's at pH 7.8. The shortening of the down stroke of the R, the absence of the S wave, and the peculiar shoulder merging from the downstroke of the R to the end of the T, have only occurred in these experiments when the perfusion fluids were distinctly to the alkaline side of pH 7.4. After perfusion with the solution at pH 8.0 for five minutes record 4b was taken. The shortening of the P-R interval is obvious as is also the change in form of the wave from the end of the R to the end of the T wave. The perfusion was then carried on for twelve minutes with a solution which was at pH 7.3 when it entered the tissue and at 7.5 when it was recovered. Figure 4c, taken at the end of this period, is particularly interesting for the reversal in the R wave is shown in three consecutive beats, and from this time on as long as the acid solutions were used the R waves remained negative. It seems reasonable to infer that the reversal point is at about pH 7.4 certainly it is not greater than 7.5 or less than 7.3. The value for pH 7.4 being the reversal point is borne out in other experiments as well. It is also to be noticed in the last cycle of 4c that the conduction time has altered so that the P wave lies between the S and T waves. At a pH of 6.2 all the main deflections were negative as is seen in figure 4d.

The mechanograms were added in the hope that a downstroke of the lever, indicating mechanical systole, would serve to locate electrical systole in the very abnormal records. This could have been the case even though there is certainly an error in the relatively heavy lever employed. However, there is no reason to suppose that the error would vary and so the constant error would not have interfered. The mechanical records did not prove useful, for in the acid solutions where they were most wanted they failed to record, in fact the hearts could not be seen to contract at all. The electrical variations continued in the apparent absence of the mechanical beat, and when an alkaline solution was again used, and when the electrical waves became more normal, the mechanical beats once more began to appear and to be registered.

The same type of electrical reversals can be obtained by using CO_2 instead of a mineral acid. The effects produced by CO_2 come on much quicker and persist for a longer time, probably because of the more rapid penetration of CO_2 and therefore the greater internal change in the cells. Haywood (1927) has made similar observations regarding the speed of the effects following the use of CO_2.

These findings lead us to a discussion of the type of records which are interpreted as "ventricular preponderence." To speak of a preponderance in regard to a turtle heart where there is only one ventricle would seem a misnomer. But when one considers that leads from the turtle heart give records of electrical variations which are similar in form to those obtained from mammalian hearts, and when the fact that the muscle bands from the mammalian heart are continuous from side to side, is remembered, then it is more than probable that "preponderance" cannot be interpreted as a balance of one ventricle against another. It seems more likely that it should be interpreted as balance of muscle areas in two or more localities. With this interpretation in mind, if contacts are placed on a turtle heart in positions relative for those of the clinical leads 1, 2 and 3, then a condition might be produced which could be comparable to that which produces the type of variation called a preponderance.

Experiments were carried out according to such a procedure. A turtle heart was perfused with Mines' solution at pH 7.8 and three contacts were placed, at the right and left sides of the base and at the tip of the apex of the ventricle. Comparison of leads one and three showed a left "ventricular preponderance" to be present in the alkaline solution. When the same solution, made acid by CO_2 to pH 4.6 was used, comparison of leads one and three showed a right "ventricular preponderance." Upon return to an alkaline solution, a left "preponderance" reappeared. Other experiments were made by injecting lactic acid into the jugular vein of an anesthetized rabbit, the standard indirect derivations being employed. In the control record a left ventricular preponderance was present and after the injection of the acid, the main deflection in lead 1 was negative, and that in lead three was positive, indicating a shift from a left to a right preponderance.

Our data have been very consistent, for in no experiment has there been a failure to reverse the direction of the waves by changing from an acid to an alkaline perfusing fluid. One record seems worthy of note in connection with the interpretation of electrocardiographic records. It is generally said that the P wave is auricular in origin, and that the Q, R, S and T waves are ventricular in origin. Eyster and Meek (1912) gave figures showing "P" waves followed by "T" waves derived from the auricle, and they suggested that the P wave probably corresponded to the QRS of the ventricular complex. Mines (1913) and more recently

Fredericq (1925) have contended that all three waves occur in both auricular and ventricular tissue. Gilson (1927) finds monophasic action curves in ventricular strips, and concludes that "the usual diphasic curve may be considered as the expression of the algebraic sum of two such monophasic action potentials at lead off electrodes plus a certain amount of modification due to extrinsic effects at other parts of the strip. Figure 5 is taken from a very slowly beating heart where an a-v block was present. By direct inspection two beats of the auricle could be counted for one of the ventricle. The mechanical beat in this record is complicated by the periodicity of the lever and so only the deeper downstrokes of the lever, indicating ventricular systoles, are of any value. One electrode was placed on the right auricle and the other on the tip of the ventricular apex. It is seen that each auricular complex consists of a series of waves which can be separated into the usual P, QRS and T groups. In the first cycle of figure 5 this group of waves is encircled by a white line. The large R, the tiny S, and the positive T of the ventricular beat are easily identified; records shown in figure 3 from an isolated auricle also present a complex series of waves for the auricular beat.

It will be noticed from the tracings obtained when an acid fluid was employed that the amplitude of the whole tracing was reduced. When these records were taken it was never necessary to alter the direction or amount of the compensating current but there was a change in resistance so that the string had to be used at a deflection value less than that used in the beginning or else had to be loosened to bring it back to the original value. Upon return to an alkaline fluid the resistance again decreased.

Mines (1913) suggested that a local acid condition might be the explanation for the condition called alternation. This conception seems to agree with our findings for in some experiments a definite alternation was recorded by the mechanical lever when the heart was being perfused by an acid solution. The alternation disappeared when the alkaline solution was again used. In an acid solution the heart is less well relaxed in diastole and in quite acid solutions, say pH 5.0 and lower, it takes appreciably longer for an equivalent amount of fluid to perfuse through the heart than is the case for an alkaline fluid.

Throughout this series of experiments the form of the electrocardiographic tracing appears to have a definite relation to the reaction of the fluid employed. Figures 3a, 4a and 4b are all characteristic of alkaline solutions. The definite characteristic is a series of positive waves shortening of the downstroke of the R wave and a shoulderlike formation merging the usual S and T waves. Figures 2b, 3b, the last two cycles of 4c, and 4d are characteristic of acid solutions. In these tracings the main deflection is negative and in very acid solutions the wave tends to become smoothed into a negative monophasic curve. Figures 1a, 1b and 1f are considered

as intermediate conditions. This interpretation seems logical for 1a was taken without perfusion and in the absence of perfusion and oxygen the tissues must have been less alkaline than normal due to the accumulation of metabolites and especially of lactic acid. Figure 1b taken after an alkaline solution not saturated with oxygen was perfused may indicate that the tissue is still somewhat acid. It is to be noted, however, that the downstroke of R is shorter than in 1a, the S is smaller, and that the characteristic shoulder present in alkaline solutions is beginning to appear. Figure 1f taken but four minutes after the return to an alkaline solution admits of the same interpretation.

Some of these variations in electrical form, particularly the reversed preponderance and the slowed conduction, may have a clinical application. An accumulation of acid in some part of the heart muscle, which must occur if the circulation to the part is altered, might influence the form of the electrocardiogram. The same explanation could serve for shifting branch bundle block. Such local accumulations of acid could occur when the pH of the mixed blood was unaltered.

The ultimate explanation of these various phenomena is not possible at present. We are not convinced that the electromotive reversals are of the same type as were found for the frog skin (Amberson and Klein, 1927). The changes in the form of the auricular and ventricular complexes in the acid solutions may be due to slowing of conduction and therefore to an alteration in the algebraic sum of the action potentials present. This suggestion would agree with that made by Katz (1927) that the direction of the T wave depends on the duration of the activity in some local muscular area.

SUMMARY

The observations of the earlier workers that the conduction time and the direction of the T wave vary according to the reaction of the solution bathing the heart are confirmed. In addition it is also shown that the R and P waves may also be reversed and that in very acid solutions the potential difference recorded tends to assume the form of a negative monophasic wave. These changes are easily reversible and can be carried out repeatedly (3 or 4 times) in the space of an hour or two. It is suggested that there are constant forms typical of acid and alkaline conditions. Our data agree with that of Carter and Dieuaide (1926) in regard to locating the critical pH at about 7.4 rather than at the lower value found for frog skin reversals by Amberson and Klein (1927), or the chronaxie values of Fredericq (1925) or those of Lapicque and Larrier (1926). In the course of these experiments we have found, also in agreement with other workers, that a series of waves can be recorded from the isolated auricular or ventricular tissue, which simulates in both tissues the complete electrocardiogram.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XXIII. Emotional Polycythemia in Relation to Sympathetic and Medullіadrenal Action on the Spleen

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Ferrari (1897) appears to have been first to study directly the influence of emotional excitement on the blood count. In students just after an examination he found an average of 457,000 more erythrocytes per cubic millimeter than in the same students before the examination—the higher counts corresponding to the most excited, the lowest to an "indifferent and phlegmatic" member of the group. The observations (on cats) by Lamson (1915–1920) were of similar import. Struck by the high erythrocyte count in some animals, and associating with it evidences of excitement and the influence of adrenalin in causing polycythemia (Boveri, 1908; Steiger, 1912, and others), and also the experiments showing that excitement causes an increased secretion of adrenin (Cannon and de la Paz, 1911), he thought that the high counts might result from emotional disturbance acting through the adrenal glands. To test the idea he allowed cats to be frightened by a barking dog. A great increase in the number of red corpuscles resulted: in one experiment the figures rose from 10,720,000 to 14, 920,000 in five minutes and in another from 11,576,000 to 14,464,000 in five minutes. Such increases (39 and 25 per cent, respectively) were taken as clear proof of an emotional polycythemia. They failed to occur after the adrenals had been removed. Lamson attributed to the liver an essential rôle in the acute polycythemia which he observed; there was, he inferred, either a liberation of erythrocytes from an hepatic reservoir or an escape of plasma through hepatic capillaries so that the blood corpuscles became concentrated.

New light has been thrown on Lamson's data by recent developments of knowledge of the spleen. Although that organ has long been known to be muscular and to contract and expand (see Krumbhaar, 1926; Barcroft, 1926b) and although the view had been expressed that it is a reservoir for

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red blood corpuscles (Gray, 1854), its service to the organism as a means of quickly increasing the number of circulating erythrocytes and of storing them away again was not clearly appreciated until the past few years. Barcroft and his co-workers (1923–1927), Cruickshank (1926), Hargis and Mann (1925), de Boer and Carroll (1924), Hanak and Harkavy (1924), Binet and his collaborators (1926–1927), Viale and Di Leo Lira (1927), and Abderhalden and Roske (1927) have proved abundantly, by various methods, that the spleen is much larger during life than after death, that various circumstances which affect the organism cause the spleen to contract, and that when it contracts it discharges into the circulation an extra supply of erythrocytes. Among such circumstances are CO-poisoning, hemorrhage, lessening of partial pressure of oxygen (i.e., varieties of asphyxia), muscular exercise and injections of adrenalin and pituitrin. Furthermore, Hargis and Mann (1925) noted that slight affective stimuli (e.g., clapping the hands, pinching his skin, display of food) caused in the dog contractions of the spleen; and recently Barcroft and Stephens (1927) have noted that indications of anxiety and jealousy were associated with diminution of spleen volume. Do not these observations on the influence of emotional states on the size of the spleen suggest that that organ, instead of the liver, is the source of emotional polycythemia? Do the adrenal glands play an essential rôle, as Lamson thought? If not, do they play any rôle—are they capable of producing any effect on the corpuscular content of the blood? The present research was undertaken with the hope of answering these questions.

METHODS. As a rule, vigorous young cats, previously selected because of their energetic reaction to a barking dog (by erection of hairs, arching of the back, dilatation of the pupils, hissing, spitting, etc.), were used as subjects.

Most of our examinations were made on blood taken from a small cut on the edge of the ear. For the preliminary or basal count the animal must be as quiet and undisturbed as possible, a condition which is favored by acquaintance with the observer, his assistant and the natural surroundings. If the animal is excited at this time the initial count may be so high that it will differ little from other counts made later. After the first count was completed the cat was confined in a small wire cage where it was protected from injury and where its motions were so limited that the effects of muscular activity (struggle) were minimized as much as possible. Thereupon a lively dog was brought near and allowed to bark at the cat for one minute. Immediately the dog was removed and a second sample of blood was secured as soon as possible from the cat's ear. In some cases three or four additional samples were examined, from 5 to 30 minutes after the period of excitement, in order to follow the course of the polycythemia.

In a few experiments tracing the course of the polycythemia each

sample of blood after being diluted was discharged from the pipette into a small tube, in which it was kept until it could be counted. The tube was rolled until the blood was thoroughly mixed with the diluent before the drop was taken for the counting slide. To test the possible effect of personal interest on the counting the figures on the tubes were changed to letters in an order unknown to the counter. The figures for cats 201 and

TABLE 1

Erythrocyte counts of normal cats before and after one minute of emotional excitement

DATE	CAT NUMBER	ERYTHROCYTES PER CUBIC MILLIMETER BEFORE AND AT STATED INTERVALS AFTER THE EXCITEMENT					
		Before	Immediately	5 minutes	10–20 minutes	20–30 minutes	Maximum increase
							per cent
October 5	201	6,056,000	9,172,000	8,676,000		6,848,000	51.4
October 13	201	7,476,000	9,672,000		7,168,000	6,872,000	29.4
October 6	202	5,148,000	6,984,000		4,928,000	5,020,000	35.6
October 13	202	6,140,000	9,244,000		6,508,000	6,318,000	50.4
October 4	203	6,248,000	8,816,000		8,020,000	6,284,000	41.1
October 11	203	7,720,000	9,300,000		7,392,000	7,484,000	20.4
October 10	204	6,884,000	8,520,000	7,516,000		7,220,000	23.7
October 14	205	7,132,000	8,760,000	7,788,000		7,128,000	22.8
October 14	206	6,860,000	9,844,000	8,680,000		7,876,000	43.4
November 4	209	5,852,000	6,760,000				15.3
November 4	210	6,688,000	7,852,000				17.3
November 7	212	7,328,000	8,212,000				12.1
November 7	213	6,764,000	8,736,000				29.1
November 8	214	5,916,000	7,728,000				30.5
November 8	215	9,792,000	11,052,000				12.9
November 16	216	7,936,000	9,404,000				18.5
November 16	217	7,928,000	8,804,000				11.0
November 17	218	8,488,000	10,440,000				23.0
November 17	219	6,696,000	9,960,000				48.7
November 28	220	7,496,000	8,760,000				16.9
November 28	221	6,336,000	7,552,000				19.1

The high basal count of 203 on October 11 was associated with some signs of anger; the cat tried to scratch the observer when he was preparing the ear for bleeding.

202 on October 13 (table 1) were obtained under such conditions. It is seen that the maximal figures did not differ to a noteworthy degree from others in the table, and that the successive counts revealed a gradual return to a lower level.

When samples were repeatedly taken from the cat, the animal was each time carefully returned to the familiar surroundings which were not disturbing.

In a few cases heart blood was observed in order to learn whether the higher counts were general or only peripheral. For that purpose the animal was fastened to a holder and excited for five minutes before the sample of heart blood was drawn. Chloralose was then administered in order to obtain blood under quiet conditions, and a second sample was taken about two hours later when the animal was wholly under the anesthetic.

In all the tests the blood was diluted (with Hayem's solution, 0.5:100) in the same pipette and counted in the same Thoma-Zeiss chamber. Pains were taken to keep all the operations as uniform as possible.

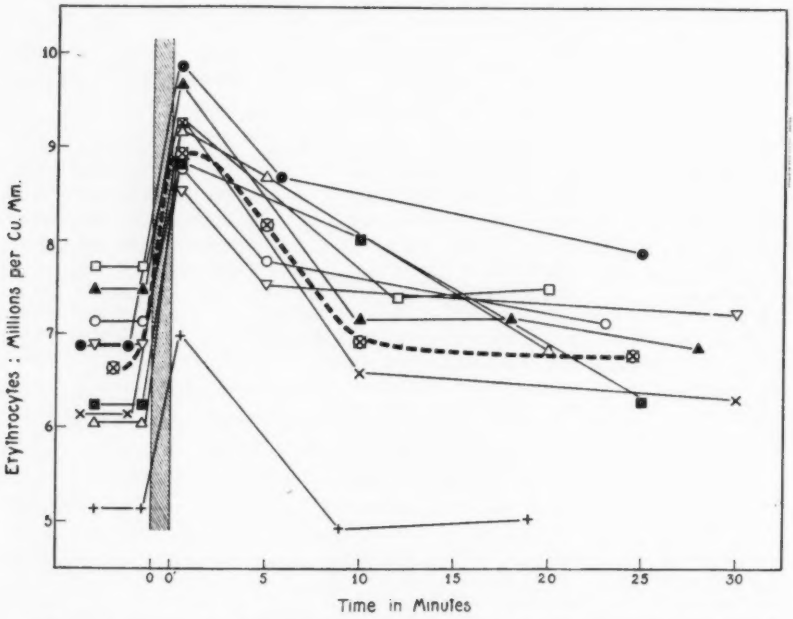

Fig. 1. The course of emotional polycythemia,—a graphic record of the first 9 cases of table 1. The vertical, shaded band represents the one-minute period of emotional excitement; the thick dash-line, the average of the 9 cases.

RESULTS. *The effect of emotional excitement on the erythrocyte count.* In 21 observations on the peripheral blood of 18 different animals the erythrocyte count immediately after excitement was invariably much higher than it was before (see table 1 and fig. 1). In 15 of the observations the increase ranged between 10 and 30 per cent above the basal level; in the remaining 6 it ranged between 30 and 50 per cent. The average increase for the series was 27 per cent; for the 15 cases with increases of 30 per cent or below it was 20 per cent.

That such increases are far beyond any variations occurring in the usual quiet existence of the animal is shown by figure 2, in which are represented graphically in the thick vertical lines the increase of red corpuscles per cubic millimeter after excitement in the first five animals of table 1, together with the results of repeated counts of blood samples taken for about a week from the undisturbed animal. With the exception of one count in the total of 27 (the first one of cat 203), the figures varied hardly more than a million corpuscles during the period. On the other hand, as a

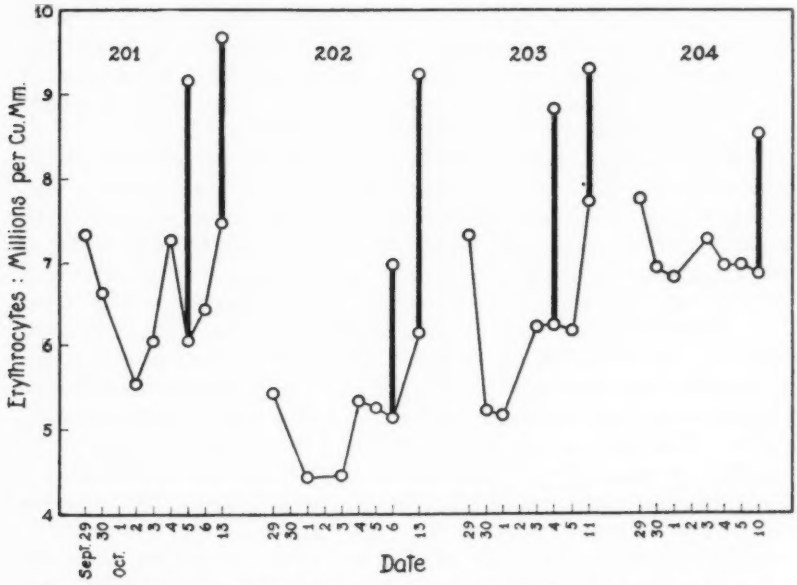

Fig. 2. Graphic record of the daily variations of the eyrthrocyte count of the first five cats of table 1, and the rises due to one minute of emotional excitement (thick vertical lines). See footnote, table 1, for explanation of the high basal count of 203 on October 11: it is noteworthy that the first counts as a rule were high.

consequence of one minute of excitement the number of erythrocytes usually rose suddenly between 2 and 3 millions.

The additional counts made at various intervals after the period of excitement showed that the maximal height of the emotional polycythemia was present as soon after the period of excitement as the blood could be collected. A fall in the numbers was notable at the end of five minutes, and within a half-hour the corpuscle count had come back to its former level (see table 1 and fig. 1).

Repetition of the excitement occasionally resulted in a gradually increas-

ing indifference of the cat, safe in its cage, to the barking dog outside. Thus cat 207, an elderly female, which had had on October 22 a blood count of 10,308,000 after excitement, became indifferent to the dog, and was undisturbed even when fastened down—a check on free motion which is usually resisted with much vigor. On November 1, after being bound to the holder and remaining there without struggle for ten minutes, the rise in the count was slight, from 5,732,000 to 6,232,000—an increase of only 8.7 per cent. It may be recalled that in previous experience in this laboratory elderly female cats were selected for x-ray observations of gastro-intestinal movements because of their serenity when held back-downward by thongs (Cannon, 1898). The absence of polycythemia corresponding to absence of excitement, in an animal which previously had manifested the concomitant presence of the two phenomena, is further testimony to the emotional origin of the high counts.

The comparative figures in the five observations on heart blood we do not stress especially because the blood samples to be compared were obtained under conditions which were different in several respects. The counts when the animals were excited were high, averaging 9,669,000 as contrasted with 8,837,000, the average figure for the excited animals of table 1. On the other hand, the counts under chloralose average lower (i.e., 6,570,000) than those in quiet unanesthetized animals (6,994,000, table 1). It would appear probable, consequently, that not only was there more than the usual increase of red corpuscles following excitement in these cases, but also a depression of the number under chloralose anesthesia. The large percentage increases may be due to both causes. In cat 205, for example (cf. table 1), the basal count on October 14 was 7,132,000, and after excitement 8,760,000—an increase of 22.8 per cent. On October 21 under chloralose the count was 6,440,000 and after excitement, 9,872,000,—a difference of 53.2 per cent. In cat 206, also, the condition was similar; the count under chloralose was 6,460,000, and after excitement 10,648,000. The comparative counts in these heart cases, therefore, seem to us not sufficiently free from complications to be strictly relied upon. They do show, however, that the increases under excitement are not due to concentration of the blood in the peripheral capillaries, but that it is general throughout the circulation.

Indications that small corpuscles are liberated during polycythemia. The larger amounts of blood obtained from the heart permitted observations to be made not only on the numbers of erythrocytes, but also on the volume of erythrocytes as shown by the hematocrit. Comparison of the percentage increases of the counts with the percentage increases of erythrocyte volumes after excitement revealed at once a marked difference. The volumes did not rise nearly so much as the counts. Observations (by the method described by Palmer, 1918) on the hemoglobin increases showed

closer correspondence of these values with the hematocrit volumes than with the counts. These results indicated a discrepancy so great that it could only be accounted for by the appearance of smaller corpuscles in the blood after excitement than were there before. Some measurements of the sizes of the corpuscles before and after excitement were made, therefore, to learn whether the microscope would reveal the presence of small sizes as the numbers increased. In table 2 are shown the size distribution of 100 red corpuscles from heart blood, that were kept in their own plasma, sealed under a thin coverglass. The size limits for cats has been given as 5–7 micra (Hayem, 1889) and 4–7.1 micra (Bethe, 1891). Lamson (1915, p. 200) calculated that after giving adrenalin and increasing the erythrocyte count about 2 million corpuscles in each of two dogs, the average volume of the corpuscles was diminished 13.4 per cent during the polycythemia. He cites a number of previous investigators who had noted that the relative increase of hemoglobin was not so great as the relative

TABLE 2

Percentage of erythrocytes of various diameters, cat 207, October 22, 1927

DIAMETERS (MICRA)	NUMBERS OF ERYTHROCYTES IN 100	
	Under chloralose	After excitement
3–4		5
4–5	10	25
5–6	60	47
6–7	30	23
Total	100	100

increase of red corpuscles (cf., e.g., Schneider and Havens, 1915; and Gregg, Lutz and Schneider, 1919). Our results harmonize with these earlier observations.

Absence of effect of emotional excitement after severance of the splanchnic nerves and removal of the upper abdominal sympathetic chains. The animals used in this test were all in excellent physical condition. Twelve observations on six different animals (see table 3) show that when the upper abdominal viscera are deprived of their sympathetic innervation, the usual emotional excitement is not followed by any prominent change in the erythrocyte count. The average count before excitement was 6,701,000; immediately after one minute of exposure to the dog it was 6,101,000; five to ten minutes later it was 6,412,000.

As a further test under these conditions, the cats were taken to the kennels where the barking dog was joined by a chorus of his fellows; the excitement continued for two or three minutes and then the cats were

fastened to the holder where they were excited for five to ten minutes more. Even with such extra stimulation the usual effects did not occur; instead the erythrocyte count fell. In cat 302, for example, the figures were as follows:

Before excitement	7,064,000
After excited by dog	6,320,000
After tied down 5 minutes	5,728,000

Effect of severing the left splanchnic and the splenic nerves, and removing the upper abdominal sympathic strands and the right adrenal gland. The effect of this operation is to exclude nervous and possible humoral (adrenin)

TABLE 3

Erythrocyte counts, before and after one minute of excitement, in cats with splanchnic nerves severed and with upper abdominal sympathetic strands removed

DATE	CAT NUMBER	ERYTHROCYTES PER CUBIC MILLIMETER			IMMEDIATE CHANGE
		Before	Immediately after	5-10 minutes later	
					per cent
October 28	106	6,660,000	5,876,000	6,192,000	−11.9
October 31	106	6,108,000	6,420,000	5,140,000	+5.1
October 26	107	7,120,000	7,088,000	6,744,000	0
October 27	107	7,500,000	7,148,000	7,376,000	−4.7
October 25	286	7,596,000	7,604,000	8,100,000	0
October 26	286	8,000,000	7,732,000	7,128,000	−3.3
October 26	302	5,876,000	5,760,000	5,244,000	0
October 27	302	5,336,000	5,440,000	4,796,000	0
October 29	303	6,596,000	6,384,000	6,108,000	0
October 31	303	6,144,000	6,424,000	6,872,000	+4.5
October 27	305	6,600,000	6,564,000	6,668,000	0
October 29	305	6,884,000	6,776,000	6,574,000	0

In this and other tables variations of 250,000 corpuscles or less in the counts are regarded as insignificant and are designated as 0.

agencies from action on the spleen while leaving the nervous system still connected with the liver through the right splanchic nerves. After the animals had fully recovered from the operation and were in vigorous health, they were excited as before for one minute. The results are given in table 4.

In three of the seven observations the figures show no increase in the number of corpuscles after the period of excitement. In the other four cases, the increases were relatively slight. Of course it may be argued that if the sympathetic strands and the left splanchnics had not been severed the liver would have had richer innervation and the results would have been different. The results obtained, however, were so little indicative of any

important rôle played by the liver that we did not take the trouble to try demedullating the left adrenal and leaving the left splanchnics. The data presented in table 4 do not lend support to the view that the liver or any other organ still innervated is of noteworthy significance in producing emotional polycythemia. This conclusion is confirmed by a considerable number of cases in which the erythrocyte count did not increase, although the nerves of the spleen were sectioned (see table 5). In these animals the splanchnic and the hepatic nerves were intact, and therefore the liver was subjected to the influence of nerve impulses and of extra secretion of adrenin as well. If the emotional increase of red blood corpuscles was due in any noteworthy degree to the liver, they should have become regularly more numerous as a result of the excitement. As table 5 reveals, this did not take place.

TABLE 4

Erythrocyte counts, before and after one minute of excitement, in cats with splenic and left splanchnic nerves severed, upper abdominal sympathetic strands and right adrenal gland removed, but with nerves to the liver intact

CAT NUMBER	INCREASE BEFORE OPERATION	DATE	ERYTHROCYTES PER CUBIC MILLIMETER			IMMEDIATE CHANGE
			Before	Immediately after	5–10 minutes later	
	per cent					*per cent*
201	29.4	October 24	7,660,000	8,056,000	8,140,000	5.1
201		October 28	7,660,000	7,992,000	7,156,000	4.3
202	35.6	October 24	6,320,000	6,728,000	6,812,000	6.4
202		October 28	6,112,000	6,100,000	5,800,000	0
203	20.4	December 7	9,944,000	9,860,000	8,924,000	0
204	23.7	October 31	7,064,000	7,648,000	7,092,000	8.2
204		December 7	9,440,000	9,360,000	9,400,000	0

Effects of sectioning the splenic nerves. A number of investigators have shown that adrenalin in small amounts causes the spleen to contract (cf. Strasser and Wolf, 1905; Hoskins and Gunning, 1917; Tournade and Chabrol, 1924, 1925). It has been observed also that adrenalin causes increase of the erythrocyte count both in lower animals (cf. Lamson, 1915) and in man (Schneider and Havens, 1915). With the spleen denervated, therefore, the possibility still remains that adrenin, set free from the adrenal medulla, would cause contraction of the spleen similar to that caused by the nerve impulses and thereby would increase the percentage of red corpuscles. To denervate the spleen the organ was exposed through a mid-line or left-side section of the abdominal wall, the arterial branches were isolated, and the small visible nerves were separated from the vessels and cut. To destroy any invisible minute nerve strands which might be left, the bared surface of the vessels was touched with a concentrated

solution of phenol. This operation was at first done on the vessels close to the spleen, but we did not always produce in this way a complete denervation, as shown by positive effects (increases from 10.4 per cent

TABLE 5

Erythrocyte counts, before and after excitement for one minute or longer, in cats with splenic nerves cut

CAT NUMBER	INCREASE BEFORE OPERATION	DATE	ERYTHROCYTES PER CUBIC MILLIMETER			CHANGE
			Before	After excitement		
				1 minute	5–10 minutes	
	per cent					*per cent*
205	22.8	October 14				
		November 3	7,376,000	7,232,000		0
		November 3	7,376,000	7,384,000		0
		November 5	6,708,000	6,892,000		0
		November 21	7,484,000	7,224,000		0
		November 23	7,728,000		8,400,000	+8.6
		December 1	8,896,000		9,024,000	0
		December 29	9,216,000		10,712,000	+16.0
212	12.1	November 28	6,116,000	6,144,000		0
		November 30	5,240,000	5,184,000		0
		December 1	4,808,000		5,608,000	+16.6
213	15.4	November 7				
		November 21	6,616,000	6,416,000		0
		November 23	6,288,000		6,272,000	0
		December 1	6,808,000		6,256,000	−8
		January 3	8,408,000		7,544,000	−10
216	18.4	November 16				
		November 21	8,936,000	9,024,000		0
		November 23	9,000,000		8,640,000	−4
		January 3	7,848,000		8,008,000	0
217	11.0	November 30	5,088,000		5,352,000	+5.2
218	23.0	November 30	9,368,000		8,352,000	−10.8
219	48.7	November 17				
		November 23	6,136,000	6,286,000		0
		November 25	5,840,000		6,552,000	+12.2
		December 1	5,640,000		5,774,000	0
		January 4	7,336,000		7,224,000	0
220	10.4	November 28				
		December 1	5,608,000		6,032,000	+7.6
221	19.1	November 28				
		December 1	5,664,000		6,840,000	+20.8

to 23.5 per cent after one minute of excitement) which disappeared after a second operation. The second operation, which we found much more satisfactory than that already described, consisted of clearing the nerves

from the splenic artery before it branched. The larger bundles of nerves are there clearly visible and can be thoroughly removed. In order to make sure that all possible neural connections were destroyed, we bared likewise the splenic vein, the gastro-splenic vessels and pancreatico-splenic artery, and, after clearing a short extent of each one, touched it with phenol.

The results of emotional excitement in these cases are shown in table 5; in all cases except one the observations were made within six weeks after the operation. Two striking features are disclosed by the figures. First, brief excitement (i.e., for 1 minute) rarely produced a noteworthy positive effect. This is in marked contrast to the results shown in tables 1 and 7. Second, longer excitement, lasting from 5 to 10 minutes, and attended by struggle, evoked occasional rises in the number of erythrocytes as great as 16 and 20 per cent, but also occasional drops nearly as great. The effects were quite inconstant and irregular; in two animals, 213 and 216, even long and very marked excitement did not yield positive results. That such displays, indeed that much less extreme displays, are attended by augmented medulliadrenal secretion is well attested (Cannon, Britton, Lewis and Groeneveld, 1927). When the normal spleen contracts under emotional conditions, therefore, two agencies are at hand and both may be operating—a greater concentration of adrenin in the blood and sympathetic nerve impulses. The experiments reported in table 5, in comparison with those reported in tables 1 and 7, indicate that in the absence of the nerve impulses the discharged adrenin is not markedly effective in causing polycythemia. This may not be due, however, to inability of adrenin to cause the spleen to contract; it may be due to greater or less failure of the denervated spleen to concentrate the blood gathered within its confines. Barcroft and Poole (1927) report that the denervated portion of the spleen yields blood which shows not more than the most trifling sign of concentration—a process possibly due to rhythmic squeezing of the plasma out of the blood and dependent on intact nerve connections—whereas the innervated portion soon concentrates its corpuscular contents. It is clear that if the blood of the spleen is not concentrated, contraction of the organ must merely add to the volume of circulating blood without augmenting the percentage of corpuscles. Most of the cases reported in table 5 are consistent with that possibility. On the other hand, in a few of the cases, when struggle and excitement were prolonged, an augmentation occurred, usually slight, but in one instance rising to 20 per cent. These cases suggest that the blood may have been concentrated in these spleens and that under such conditions the greater output of adrenin during emotional stress can cause contraction though nerve impulses are no longer delivered.

The irregular results obtained in the experiments just described might have resulted from varying degrees of excitement of the animals at differ-

ent times or from varying concentration of blood in the *denervated* spleen. These possibilities led us to test some of the same animals (and also three other animals with spleen and adrenals denervated—nos. 203, 204 and 302) for response to injected adrenalin. The amount injected was 0.02 mgm. per kilo of body weight—an amount which had been found effective and without deleterious effects in some other experiments in this laboratory. The strength of the adrenalin solution was 1:1000; it was injected intramuscularly in the thigh. The animals used were chosen from those appearing in tables 3, 4 and 5. The results obtained are shown in table 6. In two instances—in cats 203 and 205—the routine dose failed to cause polycythemia. In cat 203, however, doubling the dose developed a rise of 20.7 per cent.[2] In the other instances the injections called forth increases varying from 4 to 21 per cent. The polycythemia was slower in developing than in excited normal animals, but soon subsided. In cat 302, for example, the counts were as follows:

Before injection	5,992,000	
10 minutes after injection	7,184,000	(incr. 19.8%)
20 minutes after injection	6,392,000	
30 minutes after injection	6,024,000	

Intra-muscular injections can not be expected to have the uniform effects seen after intravenous injections, yet the variations of the figures in table 6, in response to the same doses of adrenalin, are so great as to indicate that they are due not to varying rates of entrance of the drug into the circulation and therefore not due to varying influences of the drug on the denervated spleen, but rather to varying conditions in the spleen itself. The announcement by Barcroft and Poole that the denervated spleen does not effectively concentrate its blood may account for the small and irregular effects resulting from emotionally secreted adrenin and from injected adrenalin.

Effects of inactivation of the adrenal medulla. In order to remove the influence of the adrenal medulla without disturbing the innervation of the spleen the right adrenal gland was extirpated after tying its vessels, and the medulla of the left gland was sucked out through a lateral cut by means of a sharp-edged tube connected with a vacuum pump. After complete recovery from the operation the animals were tested in the usual manner. As shown in table 7 inactivation of the adrenal medulla had no marked

[2] In cat 203, after splenectomy, a dose of 0.04 mgm. per kilo caused no assured increase; 10 minutes after injection the count was 32,000 higher than it was before. In cat 204, after splenectomy a dose of 0.02 mgm. per kilo also caused no certain increase. These results are in agreement with the conclusion drawn on p. 553 that the hyperglobulia is not due to other organs (e.g., the liver) for in these two animals only the spleen had been removed.

influence on the effects of excitement on the erythrocyte count. In three animals one minute of exposure to the barking dog increased the count between 6.5 and 28.2 per cent. The secreted adrenin is clearly not essential to the emotional effect, though that it can by itself induce an increase of

TABLE 6

Erythrocyte counts, before and after intramuscular injection of adrenalin (0.02 mgm. per kilo)

CAT NUMBER	PREVIOUS INCREASES		DATE OF INJECTION	ERYTHROCYTES PER CUBIC MILLIMETER			CHANGE
	Before	After denervation		Before injection	6 minutes after injection	10–15 minutes later	
	per cent	*per cent*					*per cent*
203	20.4	0	December 3	6,800,000	6,768,000	6,822,000	0
203			December 3*	6,800,000		8,272,000	21.6
204	23.7	0	December 8	7,888,000		9,528,000	20.8
205	22.8	0; 8.6; 16	December 1	8,896,000	9,144,000		0
213	29.1	0	December 1	6,808,000	7,444,000		9.3
216	18.5	0; 2	December 17	6,960,000		7,240,000	4.0
219	48.7	0; 12.2	December 19	5,640,000	6,392,000		13.3
302		0	December 6	5,992,000		7,184,000	19.9

* Injection of 0.04 mgm. per kilo.

TABLE 7

Erythrocyte counts, before and after one minute of excitement, in cats with adrenals inactivated by removal of right and denervation of left gland (206, Nov. 9), or by removal of right and demedullation of left (209, Nov. 7; and 214, Nov. 9)

CAT NUMBER	INCREASE BEFORE ADRENALS INACTIVE	DATE	ERYTHROCYTES PER CUBIC MILLIMETER		INCREASE
			Before	After excitement 1 minute	
	per cent				*per cent*
206	20	November 14	6,476,000	7,232,000	11.7
		November 15	6,640,000	7,072,000	6.5
209	15.3	November 12	7,164,000	8,512,000	18.8
		November 15	7,608,000	8,416,000	10.6
214	30.5	November 14	7,464,000	8,832,000	18.3
		November 15	7,960,000	10,202,000	28.2

corpuscles has been shown above. What part it may play under normal conditions remains undetermined.

Discussion. When a change occurs in the number of erythrocytes it is well to remember the relative character of the erythrocyte count. Conditions are known in which the corpuscles stagnate and become concen-

trated, especially in peripheral capillaries. Blood samples taken under such conditions (when the parts are cold, or when a state of shock or anhydremia exists) exhibit an apparent polycythemia. Is there a possibility that in our series of positive cases concentration was in part responsible for the high counts found after excitement? There might be extravasation of fluid through capillary walls, because of high intracapillary pressure, and thickening of the blood. Though this process may occur to some degree—in experiments involving struggle, for example—it is impossible to attribute to it any considerable importance in our observations. First, the most marked changes had already occurred as soon after excitement for *one minute* as it was possible to obtain the blood. Even if intracapillary pressure had increased, the interval between the occasion and the effect would probably be too short to permit any considerable transudation. Again, the animals with denervated spleens, when excited for the same length of time, manifested no increase of the erythrocytes—indeed, when excited for a much longer time in most instances they failed to do so (see table 5). If emotional hyperglobulia were to any noteworthy degree dependent on escape of plasma, some indication of it should have appeared in these tests. The absence of change confirms the inference that the blood is not thickened by transudation.

The points made in the foregoing paragraph bear on the experiments and conclusions of Lamson (1915–20). As previously stated, Lamson argued that occurrences in the liver explained the polycythemia which he observed after emotional excitement and after giving adrenalin. Either liberation of red corpuscles stored in the liver or passage of plasma through the walls of hepatic capillaries could, as he thought, produce the effects. In accounting for the differences between Lamson's observations and conclusions and our own we should recognize, first, that most of the experiments on which he based his claims were performed under anesthesia, and second, that he administered very large doses of adrenalin (e.g., 0.9 mgm. per kilo, intravenously), far beyond the physiological range. The excitement of being anesthetized and the effect of an anesthetic on the nervous organization of the body must profoundly disarrange so sensitive a response as that described in the foregoing pages. And as Edmunds and Stone (1924) have pointed out, large doses of adrenalin will cause concentration of the blood in animals with the liver excluded from the circulation. Under the natural conditions of our experiments, the hyperglobulia regularly occurred after a period of emotional stress (see table 1 and fig. 1), it occurred to only a relatively slight extent or not at all when the liver or both the liver and the adrenal glands were still innervated, but the spleen had been denervated (see tables 4 and 5 and p. 552). From these results we have had to conclude that the liver is not a significant factor in emotional hyperglobulia.

The above considerations, taken together with the evidence that the remarkably sharp rise in the erythrocyte count which normally accompanies excitement fails to appear when the spleen is denervated, point to that organ as the immediate cause of polycythemia. The spleen is not provided with arrangements for producing the effect by removing plasma from the circulating blood. It is a reservoir of red corpuscles. The question arises as to whether blood stored in the organ could account for such increases of the erythrocyte count as we have reported.

It must be admitted at the outset that the data for a thoroughly satisfactory answer to this question do not exist. By a study of the changing area of the cat spleen Barcroft and Stephens (1927) computed that the weight of the organ at rest was 34 grams and after exercise was 14.8 grams. The *post-mortem* weight was 8.57 grams—a figure corresponding fairly closely to that (8.7 grams) given by Voit (1866) for a cat weighing 3100 grams and to observations which we have made. According to the computation by Barcroft and Stephens, the spleen discharged 20 grams of its contents. It might have discharged, however, 25 grams (34 grams − 9 grams). There is evidence that the spleen can contain and expel much more blood than these figures indicate,—indeed, as much as eleven times its own empty weight. Scheunert and Krzywanek (1926), who observed increases of 25.6 and 38 per cent in the erythrocyte count of the horse after work for 5 minutes, state that whereas the corpuscles constitute 29 per cent of the circulating blood they constitute 61 per cent of the blood added by the spleen, i.e., the splenic blood is about twice as rich in corpuscles as the blood elsewhere in the body. If now we assume that the *extra* corpuscles in the spleen are stored there and when the organ contracts they are added to the circulating blood, we may consider the events as follows. The spleen discharges, we may suppose, 25 grams of its blood. If 60 per cent is corpuscles there would be 15 grams of them and 10 grams of plasma. This plasma and about 4 grams of the corpuscles may be regarded as that part of the circulating blood which is passing through the spleen, leaving 11 grams of extra, stored corpuscles. When these are added to the circulating blood, they increase the corpuscles 20 per cent. Before the addition, therefore, the circulating corpuscles amounted to 55 grams. On the assumption that this was 30 per cent of the blood in circulation, the amount would be 183 grams. If we regard the blood weight of the cat as 6 per cent of the body weight (see Erlanger, 1921), the animal would weigh 3050 grams. Most of the animals studied by us weighed at least 3000 grams, and some weighed as much as 4000 grams. Since the splenic size and capacity probably increase with the size of the body, the figures given in the foregoing estimate seem not to stretch unduly the range of probabilities.

Two qualifications should be mentioned with regard to the above dis-

cussion. First, the average increase of the corpuscles in our study by 20 per cent was an increase in their number and not in their weight. If a large percentage of the stored corpuscles liberated from the spleen are smaller than those usually found in the blood, the spleen might discharge a smaller weight of corpuscles than is calculated above and yet increase the erythrocyte count quite as much. Second, though we assumed that the splenic blood is twice as rich in corpuscles as is the blood in general circulation, that figure is not admitted by all investigators. Barcroft and Poole (1927), for example, present hemoglobin values indicating that splenic blood is only 50 per cent richer than the ordinary. Perhaps these two considerations may largely balance each other. In any event, the normal resting spleen appears, on the basis of present knowledge, to be able to hold sufficient blood sufficiently concentrated, and on occasion to contract to a sufficient degree to allow increases of about 20 per cent in the circulating erythrocytes to be explained as due to splenic discharge.

How to account for the large exceptional increases of 50 per cent, mentioned on p. 548, is a puzzle. They may be instances of accidental concentration of corpuscles in the ear capillaries, or they may indicate possibilities of splenic function beyond those now being regarded as limiting. For the present we report the results and offer no explanation for them.

Previous investigations from this laboratory have emphasized the emergency functions of the sympathico-adrenal mechanism. It is operated in emotional excitement, in physical exertion, in cold and in asphyxia, and the bodily changes which are induced thereby may reasonably be interpreted as adjusting the organism to the conditions which then prevail or are then likely to prevail. Prominent among the adjustments are those for muscular activity, chiefly the provisions for increased oxygen delivery to the active parts. It is clear that the hyperglobulia described in the foregoing pages fits into the general scheme of sympathico-adrenal adaptation of the organism to meet emergencies.

SUMMARY

Emotional excitement (of a cat in the presence of a dog), for one minute, is quickly followed by a pronounced increase of the red corpuscles of the blood. In fifteen observations of increases below 30 per cent the average increase was 20 per cent (see fig. 1 and table 1).

The increases are far beyond the variations occurring in the usual quiet existence of the animal (see fig. 2). The maximal increase is immediately after the minute of excitement; after five minutes a fall has started; and within a half-hour the former state has been restored (see fig. 1).

If the conditions which usually cause excitement fail to do so, the polycythemia does not occur.

Hematocrit and hemoglobin determinations do not show such great

increases as does the erythrocyte count; this is explained by a larger percentage of small corpuscles in the blood after excitement (see table 2).

Emotional polycythemia fails to occur after removal of the upper abdominal sympathetic strands and bilateral severance of the splanchnic nerves—indeed it may be replaced by a lower erythrocyte count (see table 3).

If only the liver among the upper abdominal viscera is left innervated, excitement causes no noteworthy hyperglobulia (see table 4).

After section of the nerves to the spleen excitement for one minute does not produce polycythemia; prolonged excitement with struggle may occasionally increase the erythrocyte count (see table 5). The adrenal glands were active. The irregular effect of secreted adrenin and of injected adrenalin (see table 6) are attributed to variable concentration of the blood in the denervated spleen.

Inactivation of the adrenal medulla has no marked influence on emotional polycythemia (see table 7).

The relations of the foregoing data to researches by other observers on the liver and on blood concentration are discussed; the possibility of an addition of 20 per cent to the circulating corpuscles by splenic contraction is also considered.

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VISCOSITY OF THE BLOOD IN HISTAMINE SHOCK

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In a previous work (Waud, 1927a) it was found that immediately following the injection of peptone or the provocative dose of antigen there was a reduction in the viscosity of the blood. As the mechanism of action of these substances is thought by some to be similar with that of histamine the present work was undertaken to determine if similar changes in the physical state of the blood occur on the injection of the latter substance.

Experimental methods. The viscosimeter used was that described by Waud (1927b); this type of instrument enables one to make repeated determinations without taking the blood from the animal. The instrument consists essentially of a glass bulb, which when in use, communicates by means of a metal cannula with the carotid artery and by a glass capillary tube with the external jugular vein. Blood flows into the bulb from the carotid artery and returns to the animal by way of the capillary tube. By clamping the artery and subjecting the blood in the bulb to an air pressure equal to that of the blood pressure of the animal the viscosity of the blood may be determined by the time taken for the blood in the bulb to be driven through the capillary tube into the vein. Determinations were made approximately every 30 seconds throughout the experiments.

Dogs under barbital and ether anesthesia were used in all experiments. Blood pressure was recorded from the femoral artery, and the left external jugular vein cannulated for injection of anticoagulant and histamine.

After injecting into the animal sufficient heparin or novirudin to render the blood incoagulable a number of viscosity determinations were made as "normals." Then 0.5 gram of histamine per kilo of body weight was either dissolved in 12 cc. of 0.9 per cent NaCl and injected into the external jugular vein, or the same quantity in dry powder placed in the bulb of the viscosimeter and the blood allowed to mix with it. Immediately after the histamine entered into the circulation viscosity determinations were made continuously until the blood pressure had returned to normal or until the death of the animal.

In one group of experiments defibrination and reinjection of the blood was used to prevent coagulation of the blood in the instrument.

Results. Typical results of 28 experiments are shown in figure 1. It will be noted that immediately following the injection of histamine there is a fall in the viscosity of the blood; this was found in all experiments except two in which no change was noted. It was found that the condition of the viscosity of the blood following the brief period of low viscosity is determined by the extent to which the blood has been rendered incoagulable. If the defibrination was carried to the point where further withdrawal of blood did not decrease the amount of fibrin obtained, the viscosity after remaining below normal for about two minutes returns to normal and no further change takes place; if however the defibrination has

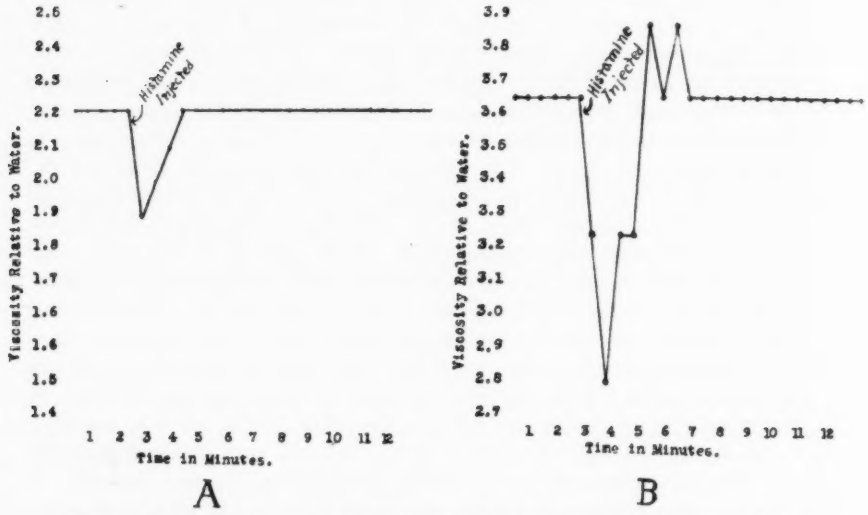

Fig. 1. Changes in the viscosity of the blood on injection of histamine. *A*. Extensive defibrination of the blood. *B*. Moderate defibrination.

not been carried out to the above extent the viscosity not only returns to normal but mounts above normal for a short time and then returns to normal and remains there. In figure 1, *A* and *B* represent the above two conditions respectively. The degree to which the blood was rendered incoagulable by the injection of novirudin or heparin had similar effect upon the viscosity of the blood.

In all experiments there was a relation between the degree of defibrination and the "normal" viscosity of the blood, extensive defibrination always resulting in a low viscosity.

Not only was the viscosity of the blood decreased by defibrination but

it was also found that as the fibrin was removed there was a progressive fall in blood pressure.

Novirudin in sufficient quantities to cause incoagulability of the blood was found to have a lowering effect upon the blood pressure.

DISCUSSION. The results of these experiments support the view advanced by the writer (Waud 1927a) that a sudden decrease in the viscosity of the blood may be a factor in the production of the fall in blood pressure in conditions of shock.

The experiments clearly show that neither defibrination nor injection of heparin or novirudin will prevent the fall in blood pressure in histamine shock.

The lowering effect of defibrination upon the viscosity of the blood and upon the blood pressure suggests that fibrinogen or some other coagulable element is a factor in the maintenance of blood pressure. It is possible, however, that other factors such as breaking up of the blood cells may be responsible for part of the fall.

SUMMARY

Experiments were performed in which repeated viscosity determinations were made before and following the injection of histamine.

It was found that the fall in blood pressure in histamine shock was accompanied by a reduction in the viscosity of the blood.

Rendering the blood of the animal incoagulable by defibrination, injection of heparin or novirudin does not prevent the fall in blood pressure in histamine shock.

The viscosity of the blood and also the blood pressure decreases with defibrination.

The author is indebted to Dr. A. J. Carlson for his direction and criticisms in this work.

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THE EFFECT OF INSULIN ON THE BLOOD SUGAR OF FISHES

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Although the action of insulin on cold blooded vertebrates has been studied, there appear to be few recorded observations of the production of hypoglycemia in fishes. More is known concerning the effect of temperature on the time required for the insulin to act than about the effect of insulin on the blood sugars. Huxley and Fulton (1924) have described the rate of action of insulin injected into frogs kept at different temperatures. They found that it required from 120 to 144 hours to bring on convulsions at 7°C., while at 30°C. all were dead within 14 hours. They did not determine the blood sugars. Olmsted (1924), working with catfish and frogs, also found that the time required for insulin to act was much less at high than at low temperatures. He made no attempt to study the blood sugars of the insulinized fishes, but did determine the blood sugars of the frogs. The frogs were kept at abnormally high temperatures to produce a condition of hyperglycemia before being injected with insulin. The insulin had no pronounced effect upon the blood sugar of frogs under such conditions. On the other hand, Hemingsen (1925) found a marked reduction in the blood sugars of two species of frogs following insulin injection.

McCormick and Macleod (1925), working with a marine fish, the sculpin (Myoxocephalus), studied the action of insulin under conditions of asphyxial hyperglycemia; and although the blood sugars of the uninjected fishes were slightly higher than those of the injected individuals used, they judged the hypoglycemic action of insulin to be comparatively feeble in fishes. Simpson (1926), in confirmation of the work of McCormick and Macleod, concluded that insulin does somewhat retard the rise of blood sugar in sculpin previously subjected to asphyxiation.

In the experiments reported here analyses were made, not only for blood sugars, but also, in some cases, for inorganic phosphorus, non-protein nitrogen, and hemoglobin, as a check on possible changes that might occur in the blood volume.

Experiments on the marine fishes were carried on in the Laboratory of the United States Bureau of Fisheries at Woods Hole. Those on trout were performed at Madison, Wisconsin, with fishes furnished by the State

Conservation Commission. The writer wishes to express his appreciation of the many helpful suggestions and criticisms of Drs. A. S. Pearse, H. C. Bradley and F. G. Hall.

MATERIALS AND METHODS. The fishes used in the experiments consisted of brown trout, *Salmo fario*, L., from fresh water and three species of marine fishes: scup, *Stenotomus crysops*, L.; menhaden, *Brevoortia tyrannus*, Latrobe; and the puffer, *Spheroides maculatus*, Schneider. An attempt was made to use the yellow perch, and although convulsions were easily produced by injections of insulin, difficulty was experienced in obtaining sufficient blood for analysis.

The trout were all males obtained from the Wisconsin State Fish Hatcheries during the winter soon after the breeding season. They were kept in the laboratory throughout the experiments in large concrete tanks through which lake water at 4°C. was continually flowing. Trout were found to be excellent fishes for experimentation during the winter, as they habitually live in cold water and are normally active during the winter months. The marine fishes were obtained from fish traps in the vicinity of Woods Hole and during the period of experimentation were kept in running sea water in hatchery boxes, one fish to each box. No experimentation was conducted for several days after the fishes were obtained in order to insure complete recovery from any asphyxiation incidental to handling.

Standard methods were employed in the analyses of the blood constituents. The Folin-Wu methods were used in determining the blood sugars and non-protein nitrogen. The hemoglobin of the trout was obtained by means of a Dare hemoglobinometer. The phosphorus was determined by Briggs' modification of the Bell-Doisy method. In the tables the hemoglobin is given in per cent in which 100 per cent is equivalent to 16.92 grams of hemoglobin per 100 cc.; the other constituents, in milligrams per 100 cc. of blood. The insulin used was prepared by the Eli Lilly Drug Company of Indianapolis.

Whole blood was used in all determinations; and lithium oxalate, 1 mgm. per cc. of blood, was used as an anticoagulant. With the exception of the puffers, the blood was obtained from the caudal vessels by cutting off the tail. The normal blood of the puffers was secured from the heart by means of a hypodermic needle.

RESULTS. *Trout.* Twenty-three trout were used, seventeen of which were given insulin, and six used for determinations on the normal blood. The trout weighed approximately one and one-half pounds each; and the dose given was in all cases 0.5 cc. of 20 unit insulin which produced convulsions in all but two fishes. No attempt was made to determine the minimum dose required to produce convulsions, but the indications are that a stronger dose, per unit of body weight, is necessary for cold-blooded

than for warm-blooded animals. The temperature of the water was maintained at 4°C. The length of time required for convulsions to occur at this temperature was very variable, ranging from less than 24 up to 60 hours, with an average of 36 hours. Most of the convulsions occurred the second day following the injection.

The convulsions produced were very similar in character to those observed by Olmsted (1924) in catfish, following the injection of insulin. Preceding the convulsions of the trout they became "nervous" and were easily excited. The skin became very dark in color, and in a few cases appeared to be entirely black. During the convulsions the fishes were unable to maintain their balance, swimming in spirals, and some assuming a vertical position with heads downward. At times the fishes would lie on their sides with mouths opening and closing; then suddenly they would dart through the water swimming in circles or spirals, after which they would again lie on their sides for several minutes. Two fishes developed no convulsions, although for two days they appeared to be "nervous," and would dart about the tank on the slightest stimulus. It is possible that these two fishes had convulsions during the night, and had recovered by the next day; but this does not seem likely since all others died from insulin shock unless glucose was administered.

Convulsions were allowed to continue with some of the fishes until death, which occurred in from 2 to 4 hours after the beginning of the convulsion (table 2). Others were injected intraperitoneally with 3 cc. of Locke's solution containing 10 per cent glucose. In these cases convulsions ceased within a few minutes and the fishes appeared to be normal again. In the present experiments, as in those of Huxley and Fulton (1924), working with frogs and of Olmsted (1924) with catfish, the injection of glucose gave only temporary relief, the convulsions again appearing after an interval varying from 6 to 48 hours. If glucose was not administered during the second convulsion, the trout died. Permanent relief was obtained in some of the fishes following the second injection of glucose; but others had convulsions a third or even a fourth time, and required further injections of glucose. None of the trout required more than four glucose injections to give permanent relief, but all required at least two. The second, third and fourth convulsions appeared to be as violent as the first.

Blood sugars were determined at various intervals following the injection of insulin. The blood on being removed from the fishes having convulsions was very dark in color, and resembled the dark blood of asphyxiated fishes. The blood sugars of six normal trout, in milligrams per 100 cc. of blood, were 75.7, 66.6, 71.9, 62.7, 86.9 and 88.8, respectively, an average of 75.4 (table 1). Five trout bled during convulsions showed blood sugars values of 23.6, 23.3, 23.3, 30.5 and 26.3, an average of 25.4

TABLE 1

Blood constituents of normal trout 4°C.

NUMBER	SUGAR	HEMOGLOBIN	NON-PROTEIN NITROGEN	PHOSPHORUS
	mgm.	*per cent*	*mgm.*	*mgm.*
1	75.7	43.0	36.1	8.6
2	66.6	40.0	29.0	8.8
3	71.9	63.5	35.2	9.4
4	62.7	65.0	35.0	8.7
5	86.9	52.0	40.5	8.8
6	88.8	60.0	27.4	7.6
Average.....	75.4	53.9	33.9	8.6

TABLE 2

Effect of insulin on trout 4°C.

NUMBER	REMARKS	SUGAR	HEMOGLOBIN	NON-PROTEIN NITROGEN	PHOSPHORUS	HOURS FOR CONVULSIONS	NUMBER OF GLUCOSE INJECTIONS
		mgm.	*per cent*	*mgm.*	*mgm.*		
1	Bled during 1st convulsion	23.6	36	24.0	8.4	59	
2	Bled during 1st convulsion	23.3	44	27.0	7.5	46	
3	Bled during 1st convulsion	23.3	49	46.7	8.7	55	
4	Bled during 1st convulsion	30.5	62	46.7	9.8	30	
5	Bled during 1st convulsion	26.3	50	48.5		31	
6	Died after 1st convulsion					60	
7	Died after 1st convulsion					24	
8	Died after 1st convulsion					24	
9	Bled during 2nd convulsion	27.4	75	44.2	5.8	25	1
10	Died after 2nd convulsion					25	1
11	Died after 4th convulsion					26	3
12	Recovered (2 convulsions)					25	2
13	Recovered (2 convulsions)					48	2
14	Recovered (4 convulsions)					50	4
15	No convulsions in 2 weeks						
16	Bled after 12 days. No convulsions	60.8	38	33.8			
17	Bled after 24 hours. Before convulsions appeared	48.7	63	34.0	7.1		
Average of 5 bled during 1st convulsion		25.4	48	38.6	8.6		
Average time for convulsions to appear						36	

which is approximately one-third of the normal blood sugar (table 2). The blood sugars of one bled during the second convulsion, i.e., during the convulsion following the first injection of glucose, also fell below 30 mgm. One trout was bled before insulin shock had occurred, 24 hours after insulin injection, and a blood sugar of 48.7 mgm. was obtained. Of the two fishes that showed no convulsions, one was analysed 12 days after the administration of insulin, and the blood sugar value of 60.8 mgm. indicated that it was practically normal.

An attempt was made by analyses for non-protein nitrogen, inorganic phosphorus, and hemoglobin to determine if there were any changes in

TABLE 3

Effect of insulin on marine fishes. Average blood sugars in milligrams per 100 cc. of blood, 19.5°C.

	NORMAL SUGAR	REDUCED SUGAR	HOURS BEFORE CONVULSIONS
Scup:			
Number of individuals	11	6	14
Minimum	46.7	18.2	20
Maximum	68.9	21.1	26
Average	55.6	19.8	23
Menhaden:			
Number of individuals	19	2	2
Minimum	46.3	29.3	6
Maximum	108.0	32.1	8
Average	80.6	30.7	7
Puffer:			
Number of individuals	6	8	8
Minimum	31.4		
Maximum	46.8		
Average	38.6	No convulsions in three weeks	

the concentration of the blood. Hall, Gray and Lepkovsky (1926), have shown that during asphyxiation in fishes these constituents increase in concentration. In the present experiments the color of the blood resembled that of asphyxiated fishes, but there was no indication of a change in concentration of the blood following insulin injections. (Compare tables 1 and 2.)

Marine fishes. As the results of the experiments on menhaden and scup were similar to those on trout only a summary of the findings is given in table 3. Besides sugars, determinations were made on the blood of scup for non-protein nitrogen, hemoglobin, and inorganic phosphorus to

note if any changes in blood volume occurred. The results here, as with the trout, were negative, and the details are thus omitted.

Scup of approximately the same weight were chosen, the average weight being 220 grams. Intraperitoneal injections of 10 units of insulin were given to fourteen of these fishes which were kept in the hatchery boxes at 19.5°C. Convulsions appeared in about 23 hours after the administration of insulin. The blood sugars of six insulinized fishes were determined, and their values in mgm. per 100 cc. of blood were 18.8, 20.8, 21.1, 20.2, 18.2 and 19.6 respectively, an average of 19.8. Eleven normal individuals gave blood sugar values between 46.7 and 68.9 mgm., the average being 55.6 mgm. Four scup, in the midst of convulsions, were given injections of 4 cc. of 5 per cent glucose, and all received permanent relief. Only one injection of glucose was necessary. Four others receiving no glucose died within two hours after the convulsions began.

Two menhaden were each given 15 units of insulin at 19.5°C. Convulsions occurred in 6 and 8 hours, with blood sugars reduced to 32.1 and 29.3 mgm. respectively. The average normal blood sugar of menhaden as obtained from 19 individuals was found to be 80.6 mgm. Thus with menhaden, as with scup and trout, a marked reduction of the blood sugars resulted from the insulin injections.

Eight puffers were given insulin injections of doses varying from 8 to 15 units, and some were given several injections at varying intervals over a period of three weeks. In no cases were convulsions produced, although the dose of insulin was large considering the relatively small size of the fishes. Puffers have relatively low normal blood sugars, whose values mostly fall between 30 and 45 mgm.

Discussion. The results of these experiments clearly indicate that in fishes with relatively high normal blood sugars the hypoglycemic effect of insulin may be readily demonstrated. In trout, scup and menhaden, all relatively active fishes, the comparatively high blood sugars were reduced by insulin injections to approximately one-third of their normal values. On the other hand in the sluggish puffer, with its relatively low blood sugar, insulin appeared to have no effect. Convulsions did not develop in the puffer, although repeated injections of insulin were given. McCormick and Macleod (1925), concluded from their work on sculpin that the hypoglycemic action of insulin on fishes was comparatively feeble. The sculpin, like the puffer, has normally a very low blood sugar content (10 to 30 mgm.); and in both of these fishes the normal sugars are often even lower than the reduced sugars of the insulinized trout, scup and mehaden. Though the evidence is limited, it suggests the possibility that sluggish fishes with a relatively low blood sugar content have a different method of controlling their sugar metabolism than those with relatively high blood sugars.

It seems that the action of insulin is dependent upon the metabolic rate. Huxley and Fulton (1924), state that ". . . . the activity of insulin itself is not essentially altered by temperature, but its speed of action is dependent upon the metabolic rate of the animal itself." They further state that if one plots the rate of action of frogs (reciprocal of time to convulsions), at various temperatures against the corresponding curve given by Krogh (1914) for oxygen consumption, one finds that they coincide in a most surprising way. Olmsted's (1924) work agrees with that of Huxley and Fulton.

The average time for convulsions to appear in the trout at 4°C. was 36 hours, while Olmsted (1924) reports that in catfish kept at 21°C., 51 to 57 hours were required for the appearance of convulsions. This at first may seem in contradiction to the supposition that the speed of action of insulin is dependent on the metabolic rate. However, if one considers the habits of the two fishes, this seeming inconsistency does not materialize, for the trout normally live in cold water, and those used in these experiments were obtained from the State Fish Hatchery where the temperature of the water in the brooks, even during the warmest part of the year, does not rise above 8°C. It seems probable that the metabolic rate of the trout at 4°C. is greater than that of the sluggish catfish at 21°C. Also, menhaden developed convulsions in from 6 to 8 hours following insulin injection, while scup at the same temperature required about 23 hours. Menhaden are more active fishes than scup, and their rate of metabolism is undoubtedly greater. It would seem that these results add further evidence in support of the supposition that the metabolic rate of the animal itself is of more importance than the influence of temperature in determining the effect of insulin on the poikilothermal vertebrates.

One cannot watch cold-blooded animals during insulin convulsions without feeling that there is interference in some way with respiratory function. The behavior of fishes reported here and of several others not reported, seemed to indicate great difficulty in getting enough oxygen during the late stages of convulsions. There was continual gaping similar to that shown by fishes when dying of asphyxiation. Necturi, after injections of insulin, move their gills at a much faster rate than uninjected ones in the same aquarium, and spend much of their time with their nostrils out of water. Moreover, they often gulp air. The extreme dark color of the blood, resembling the dark blood of the asphyxiated fishes, also suggests that the tissues are not getting a normal supply of oxygen.

Packard (1907) has shown that by injecting various sugars into the body cavity of Fundulus much greater resistance to lack of oxygen is afforded, thus indicating that sugars are an aid in respiration. If sugar injections increase resistance to lack of oxygen, it seems probable that reduction of blood sugars by insulin injections may interfere with the fishes getting an adequate supply of oxygen.

SUMMARY

Following injections of insulin, convulsions were easily produced in trout, menhaden and scup. The blood sugars of these fishes were reduced to approximately one-third the normal value.

Convulsions were not produced in the puffer although injections of varying doses of insulin were given. Though the evidence is limited, the possibility is suggested that sluggish fishes with low blood sugars have a different mechanism of sugar control than active fishes with high sugar content.

Permanent relief from insulin convulsions was obtained by administration of glucose. At least two injections of 10 per cent glucose were required to give permanent relief in the trout, while one injection of 5 per cent glucose was sufficient in the scup.

Evidence is added in support of the hypothesis of Huxley and Fulton, that the rate of action of insulin is dependent, not upon temperature alone, but upon the metabolic rate of the animal.

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THE ELIMINATION OF HEMOGLOBIN BY THE KIDNEY

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The work of Adami (1) in injecting laked blood intravenously into a dog and later examining the unstained sections of the kidney to find the site of the elimination of hemoglobin, was referred to by Cushny (2) as an excellent method of attack, on the problem of kidney functioning, that had not been pursued.

Since the possibility of tracing a colored protein through the process of being eliminated seemed to offer considerable advantage over the usually employed method of injecting various dyes, the work of Adami was repeated and extended.

METHOD. Four dogs were used in the experiment. A quantity of blood, varying from 40 cc. to 90 cc., depending upon the size of the dog, was withdrawn from the femoral artery into an equal quantity of distilled water, defibrinated, filtered and reinjected into the femoral vein of the same dog from which it was withdrawn. In each case barbital-sodium was used for anesthesia and a little ether was given when the operation was begun. Both ureters were cannulated and samples of urine containing hemoglobin were obtained and kept for examination.

The kidneys were removed at various stages in the process of the elimination of hemoglobin, one at the first appearance of hemoglobin in the urine, one when the elimination was practically complete and the others at various intermediate stages, so as to have kidney material obtained at all stages in the process of elimination.

In dog A one kidney was removed, for control, before the injection of the laked blood was made, and the other was removed thirty minutes after the urine first showed an appearance of hemoglobin, which was one hour after the injection was made. Slides were prepared from both these kidneys from blocks fixed in Zenker's solution, embedded in paraffin and stained with hemotoxylin and eosin. A part of the slides was left unstained as a check on the possibility of the hemoglobin being washed out in the process of staining. Since eosin stains hemoglobin a distinctive orange yellow color, and therefore was sufficient for the purpose, all the remaining slides were stained with it alone.

In dog B 42 cc. of blood were withdrawn and reinjected as previously

described. One kidney was removed two and a half hours after hemoglobin first appeared in the urine and the other kidney was removed one hour later. A part of the material was treated as in dog A and a part stained *en bloc*, then embedded, sectioned and mounted after only a few drops of xylol had been added to dissolve the paraffin thus eliminating the possibility of washing out the hemoglobin from the capsules.

In dog C the procedure was the same as in dog B with the exception that as soon as hemoglobin appeared in the urine, the left renal artery and vein were clamped off and a part of the kidney was removed, from the posterior pole, for fixation. This procedure was used to minimize the effect of the operation upon the other kidney. After urine formation began again in the other kidney it was removed and parts of it were fixed and both samples of tissue were treated as in dog B.

In dog D the left kidney was removed one hour and thirty minutes after hemoglobin appeared in the urine. The right kidney was not used for it had not begun to secrete again after three hours.

Examination of the urine. Macroscopically the urine samples varied from slightly red to intensely so, but under the microscope in even the most concentrated samples, while they looked yellower than the other samples or than normal urine, it would be impossible to say from a microscopic examination alone that the kidney was secreting hemoglobin. In no case were erythrocytes found in the urine. Upon examination with the spectroscope, however, the urine gave the characteristic dark bands of oxyhemoglobin. In some cases the samples were so concentrated it was necessary to dilute them to as much as twice their volume to prevent the entire spectrum from being absorbed.

Microscopic examination of the slides. In all the unstained slides, including the normal ones from dog A, there was an iridescent border, apparently chromatic aberration, along the edges of the capsular walls, presenting an appearance that Adami interpreted as the presence of hemoglobin. Apparently he did not section normal kidney tissue to test this conclusion.

In the stained slides, both in those prepared in the usual method and in those stained *en bloc*, as previously described, there was no hemoglobin found on the capsular walls nor in the clear space between them, except in three cases out of over eleven hundred capsules studied. In these three instances undoubtedly the hemoglobin had been carried there by the sectioning knife.

On the other hand in all cases, except the normal, there were some tubules in every section that showed hemoglobin. In dog A there were traces of hemoglobin in the tubules. In dog B hemoglobin was found in many of the tubules, as was the case also with the tissue from dogs C and D, the concentration of hemoglobin being highest in the left kidney of dog B, it having been removed apparently at the height of elimination.

CONCLUSION

1. The iridescent appearance around the edges of the capsules as observed, in sections from which the embedding material had not been removed is not hemoglobin; first, because when stained with eosin it does not give the characteristic orange yellow color of hemoglobin, and second, when viewed with an achromatic lens it is not longer visible.

2. The inability to find hemoglobin in the capsular space does not mean that it is not filtered through there, for even if the filtration reabsorption theory is correct, the hemoglobin would be in the capsular space in very dilute form, and as "magnification is dilution" the possibility of finding it there by this method is doubly improbable.

3. The finding of large quantities of hemoglobin in the tubules does not argue that it is separated from the blood stream there, nor does it argue against that view.

4. The method does not give promise of being more fruitful of critical results than that of various dye injections in which the experimenter has attempted to trace through them the source of their elimination from the kidney.

The author wishes to thank Dr. A. J. Carlson for suggestions, criticism, and the use of his laboratory in carrying on this work.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

VI. SUGAR[1]

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Since it had been reported by Hammarsten (1) that sugar passes into the sweat in diabetes, it occurred to us to investigate and discover whether this constituent was also constant in sweat of normal individuals. We deemed it especially desirable since we could employ new colorimetric methods which have more recently come into vogue. The method of producing sweat was that previously described by one of us (2).

This work may be divided into two periods, the first of which was undertaken during the summer of 1926 by Silvers and is reported in table 1, while the second period covers the work of the summer of 1927 by Forster for which the results are reported in table 2. During the first period we employed the method of Folin and Wu (3) for making our determinations. At this point it is well to stress the fact that with this method we must always keep in mind that the presence of either creatinin or uric acid will introduce factors of error. If they are present they must be previously removed before this experiment can be undertaken.

While Capranica (4) has reported a trace of creatinin in the sweat, we have so far found it to be negative in our tests. We are satisfied that if it is present, it must be in such minute quantities that it can be disregarded as a source of error. While in like manner Saiki (5) in this laboratory has discovered uric acid in the sweat, but he has found it in no greater quantities than 1 to 3 mgm. per 1000 cc. of sweat. Again as this occurs in such small quantities, we believe that we are justified in also disregarding this as a possible source of error.

During the first period we were able to make 27 different tests on 11 subjects, sugar being found in the sweat in every instance varying from 5.6 to 40 mgm. per 100 cc. of sweat.

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We also made simultaneous tests for sugar in the blood filtrate. A control sample of blood was taken just before and another immediately after sweating. In the twenty-eight blood tests there will be noted a fall in the blood sugar after sweating in 21 of the cases, while in 6 there is a rise and in one there is no change.

TABLE 1

The simultaneous determination of sugar in sweat and blood

SUBJECT	SWEAT	BLOOD 1	BLOOD 2
G.		121.9	105.8
F. C.	6.27	189.5	143.8
G. M.	9.47	131.5	108.1
R. B.	40.0	131.6	126.4
F. C.	9.0	125.0	115.6
M. A.	15.3	137.0	127.4
J. A.	29.3	76.6	114.0
H.	9.1	110.0	102.0
W. K.	8.5	132.5	114.3
I. R.	5.4	117.0	123.0
R. B.	17.0	138.0	104.0
C. H.	16.0	142.8	126.6
F. C.	5.7	110.4	144.7
J. A.	13.7	183.5	181.8
G. M.	14.6	146.0	109.0
W. K.	12.2	132.4	125.0
F. C.	9.0	277.7	71.9
I. R.	8.9	139.8	165.0
W. K.	13.3	200.0	180.1
C. H.		96.1	114.2
J. A.	15.7	133.3	133.3
I. R.	7.2	107.5	125.0
F. C.	33.4	169.0	126.0
O. H.	11.5	126.2	92.5
J. A.	19.7	120.5	120.0
F. C.	8.1	126.6	122.0
W. K.	5.6	122.7	98.5
C. H.	22.9		
O. H.	21.3	119.5	115.8

The above figures represent milligrams per 100 cc.

It is very interesting to note that on one occasion subject F. C. showed a very high blood sugar of 277 mgm. per 100 cc. while from the sample taken immediately after there was a remarkable drop to 79 mgm. per 100 cc. A qualitative test of the urine sample taken shortly after sweating showed a strong positive sugar reaction. Upon inquiry it was discovered that on the night previous he had consumed about 2 pounds of chocolate cake in addition to a regular meal. This would account for the high

TABLE 2

The simultaneous determination of sugar in sweat, blood and urine

SUBJECT	SWEAT	BLOOD 1	BLOOD 2	URINE 1	URINE 2
F. C.	10.7	92.6	98.9		
I. R.	10.6	92.4	99.9	80.0	50.0
J. L.		103.6	109.2		
F. C.	12.2	96.0	96.3		
J. B.		93.0	116.2		
R. G.	13.1	88.7	94.0		
O. H.	20.7			105.2	125.2
O. B.	6.8	319.0	324.0	358.9	347.8
F. C.	2.8	101.0	80.7	51.6	67.8
F. H.	5.9	88.9	95.1		
A. S.	10.6	112.2	93.8	98.4	80.0
F. B.	13.1	115.6	125.0		
R. P.	8.3	93.4	118.0	58.4	125.8
I. R.	5.9	88.1	92.6		
J. L.		93.9	82.5		
J. B.	5.5	91.3	88.1	111.2	100.0
F. H.	7.6	94.3	84.7	66.0	62.6
B. J.	9.4	103.0	111.0	62.4	70.4
F. B.	10.8	79.2	82.5	54.7	59.6
O. H.	5.0	82.6	93.8	76.0	118.0
F. C.	11.2	93.0	99.5	76.9	90.8
I. R.	7.2	112.3	124.2		
B. J.		90.4	124.2	66.8	71.2
F. B.	9.6	84.7	109.0	69.2	71.2
J. L.	18.2	95.2	109.8	51.6	86.8
F. H.	9.7	82.6	88.5	88.8	97.2
O. H.	13.1	84.8	98.0	142.0	204.8
F. B.	12.5	86.9	94.0	117.6	140.0
F. C.	25.0	104.7	105.2	46.8	54.0
I. R.	16.4	108.1	106.9	66.6	61.0
B. J.	13.0	91.4	94.4	71.2	120.0
A. S.	14.4	89.2	101.0	96.0	97.6
I. R.	24.2	107.5	93.2	76.0	156.1
T.	7.3	90.9	88.1	100.0	152.2
F. B.		79.6	89.2	25.8	40.0
J. L.		83.3	85.8	56.3	100.0
B. J.	8.5	91.7	91.3	82.4	115.1
F. H.	17.7			63.6	96.0
F. C.	7.8	119.0	119.7	128.8	160.0
V.	15.4	87.7	88.1	102.4	144.0
F. C.	8.6	100.0	137.2	142.8	173.6
F. B.		91.9	133.3	71.4	82.4
F. B.	89			48.5	64.5
F. B.	12.4			96.0	145.2
A. S.	5.5			72.1	146.8
F. B.	5.6			72.1	145.8

TABLE 2—*Concluded*

SUBJECT	SWEAT	BLOOD 1	BLOOD 2	URINE 1	URINE 2
O. H.	3.4			73.2	80.8
O. H.	6.2	85.4	98.5	117.6	173.6
F. H.	12.9	82.0	89.2	148.1	148.4
B. J.	7.1	89.6	97.0	138.0	142.8
V.	6.0	87.0	96.0	122.8	160.0
T.		99.9	94.9	56.3	61.6
F. B.		140.8	200.0	43.6	60.4
F. C.	6.5	82.2	101.0	88.8	166.4
F. H.	7.7	89.6	111.1	37.6	39.9

The sweat, blood and urine are given in milligrams per 100 cc.

hyperglycemia which was alimentary in character without a doubt. It is also interesting to note that with this high blood sugar concentration, the sweat yielded only 9 mgm. per 100 cc. which is far from being high as our figures indicate.

In the second period of our experiment we not only made a simultaneous study of the sugar of the sweat and blood, but we also included the urine. It is well to state in this connection, that, as far as urine is concerned, we are reporting the total copper-reducing substance after the customary removal of the nitrogenous disturbing factors. There has arisen considerable controversy as to what if any extent this reduction might be due to dextrose. Some of the earlier workers, among whom were Baisch (7), Pavy and Siau (8), and Moritz (9), were all more or less convinced that dextrose at least formed part of the copper reducing substance. Some years later Benedict and Osterberg (10) came to the same conclusion after the determination of the total sugar and the loss produced by the process of yeast fermentation. They also maintained that "the unfermentable portion was a true sugar or at least was derived from carbohydrate metabolism."

More recent observers have contended that dextrose is not present in the normal urine. Folin and Berglund (11) maintain that "the sugar of normal urine consists of a motley variety of carbohydrate products or carbohydrate derivatives." Host (12) classifies the reducing substances as of the glucid nature of unknown composition but not as glucose. However, for the sake of simplicity, we will speak of it as *urine sugar* like that of the sweat and blood.

The first sample of urine was voided just before sweating and the second sample about 20 minutes after retiring from the cabinet. During the second period of our work we adopted the method of Benedict (6) for making our determinations which rather uniformly gave a lower reading than that of Folin and Wu. In consulting table 2 there will be noted a few

instances in which the sweat, urine and blood data are lacking. These omissions were unavoidable due to one cause or another, however, sweating was produced in every instance where blood or urine sugar is reported.

In the second period we are reporting 46 determinations on the sweat. It might be added that there are six more that are not included here. These were determined without the blood or urine tests. The total number of tests for sweat for this period then would be 52. In 48 tests it will be noted that in 38 cases, or about 80 per cent, there was an increase in the blood sugar after sweating. In a more striking manner one can observe that out of 45 urine tests, there was an increased sugar elimination in 39 cases after sweating or in about 90 per cent of the cases. In the 38 experiments where it was possible to make simultaneous comparisons of the blood and urine sugar, there were 32 cases where the sugar of the urine and blood varied more or less directly. This represents about 90 per cent of the experiments. In 28 of the above mentioned experiments there was a simultaneous increase in the blood and urine sugar after sweating.

It is well to call attention to the fact that subject O. B. was a diabetic who had been on a diet and under insulin treatment for several weeks, consequently he was sugar-free. Furthermore, 36 hours previous to the experiment he was instructed to refrain from insulin and return to a normal diet which accounts for the hyperglycemia and glycosuria noted. Further attention may be called to the rather low concentration of sugar in the sweat, for 6.8 milligrams per 100 cc. can not be regarded as high. Further than this we are in no position to draw any conclusion as to the elimination of sugar through the sweat glands of a diabetic.

We can speak with more confidence from our findings in the sweat of normal individuals, since with our data as variable as they are, we are totally at a loss to show any correlation between the concentration of sugar in the sweat and that of the blood or urine. Since we have discovered sugar in all of the 76 determinations upon 23 different subjects ranging from 2.8 to 40 mgm. per 100 cc. of sweat, we believe that we are amply justified in saying that sugar is a constant constituent of the sweat of normal and presumably diabetic subjects. In this connection a private comment of Doctor Howell is worthy of note in which he stated that the sugar in the sweat might be the cause of the added attraction of flies for man while sweating.

SUMMARY

Sweat is shown to contain sugar in the concentrations ranging from 2.8 to 40 mgm. per 100 cc.

Sweating has a tendency to increase the blood sugar concentration.

Sweating has been found to increase the sugar of urine in 90 per cent of the cases.

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Note: Since this article was sent in for publication my attention has been attracted to an article by Usher and Rabinowitch of Montreal in "Archiv. of Derm. and Syph., XVI, 706" in which they present evidence that the sugar of the sweat is dextrose and furthermore, that the rate was increased in eczema. The amount of sugar per 100 cc. confirms our findings.

THE TREATMENT OF PARATHYROIDECTOMIZED DOGS WITH COD LIVER OIL

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In view of the fact that much of the successful treatment of parathyroidectomized dogs has either directly increased the blood calcium or has tended to do so, it would seem that any measure which acted favorably upon calcium retention might be of value. Both cod liver oil and exposure to ultra violet light have been reported by different investigators to exercise a definite influence on calcium retention in rickets and other experimental conditions resulting in low blood calcium (Brown, MacLachlan and Simpson, 1920; Hess et al., 1923; Park, Guy and Powers, 1923; Hoag, 1923; Orr et al., 1924; Hart, Steenbock and Elvehjem, 1924; Swingle and Rhinhold, 1925; McAskell, Henderson and Magee, 1926).

Since a low blood calcium is a constant finding in parathyroidectomized dogs, an attempt to prolong the lives of such animals has been made by the use of cod liver oil in the diet.

While this work was in progress a report was published by Jones (1926) giving evidence of the benefit of cod liver oil when administered daily for two weeks before the parathyroidectomy; post operative treatment, however, was found of no value in preventing tetany or prolonging life.

This study was carried out upon thirty-four dogs, sixteen of which were controls. The following diet was given daily: 400 to 500 cc. of milk, 130 to 150 grams of kibbled dog cake, 20 cc. of cod liver oil (Lilly) and 250 grams of hamburger twice a week. When muscle twitching was noticed, the amount of cod liver oil was increased to 30 cc. until symptoms subsided. If food was refused 300 to 400 cc. of milk and 20 cc. of cod liver oil were given by stomach tube.

The controls developed tetany on an average of 2.5 days and the test animals on an average of 8.2 days following thyro-parathyroidectomy. In most cases tetany in the test animals was milder and the attacks less frequent than in the controls. A few of the animals receiving cod liver oil showed no more symptoms of tetany than anorexia, and mild, infrequent muscle twitchings.

Calcium estimations were made on the serum by Collip and Clark's

modification of the Kramer and Tisdall method (1925). Serum calcium dropped to the tetanic level in the test animals within four days, and in the controls within 2.5 days. After thirty to forty days, the serum calcium was practically normal in the animals given cod liver oil. At this time the use of cod liver oil was discontinued and no further tetany occurred except when estral cycle, pregnancy or infection would precipitate it. Serum calcium in the controls never reached the pre-operative level. Twenty-five per cent of those receiving calcium and 50 per cent of those receiving cod liver oil recovered completely. The animals given cod liver oil were more active, better nourished, and less subject to infection than those receiving calcium lactate in their diet.

One pregnant dog was thyro-parathyroidectomized and on the thirty-fifth day after operation delivered nine puppies. For six days following delivery she received 30 cc. of cod liver oil in addition to her regular diet, and during this time showed no tetany. The dosage was then reduced to 20 cc. and she progressed through lactation with no symptoms of tetany other than occasional stiffness in the posterior extremities.

In two thyro-parathyroidectomized animals observed during the estral cycle, blood calcium dropped to the tetanic level of 6 to 7 mgm. per 100 cc. of blood on the second or third day after the beginning of estrus. The animals refused food and were stiff at this time but were carried along successfully by giving 20 cc. of cod liver oil and 300 to 400 cc. of milk by stomach tube daily until the cycle was over.

A few animals developed such a severe gastro-intestinal upset after the operation that the cod liver oil was not well retained. However, there were fewer animals that suffered from a gastro-intestinal disturbance among those given cod liver oil than those given calcium lactate in their diet.

Five hundred cubic centimeters of milk were used in the diet of the animals reported, but the favorable results cannot be attributed to this factor since the controls received a similar amount of milk. Dragstedt and Sudan (1926) in recent work have shown that 500 to 1500 cc. of milk daily were not sufficient to prevent the appearance of tetany in adult dogs nor to preserve the life of these animals after operation. They found that even 1500 to 2900 cc. daily did not control the tetany of pregnancy or of lactation. The suggestion is made that the well-known ameliorative effect of a diet of cow's milk in parathyroid tetany is more probably due to its content of lactose than to its calcium content. They also think the reason that human milk is more effective in preventing tetany in infants than cow's milk, which is richer in calcium than human milk, is its greater content of lactose.

The average delay in the development of tetany, the amelioration of symptoms, and the consequent recovery of thyro-parathyroidectomized

dogs given cod liver oil furnishes evidence for its value when used post-operatively.

As a possible mechanism by which cod liver oil may influence parathyroid tetany, the work of Robins and Boyd (1923) should be cited wherein they showed that fats depress the tonicity of the gastro-intestinal tract. This may allow for a greater absorption of calcium and a lessened excretion. Whether the prevention of a gastro-intestinal upset in those animals receiving cod liver oil was due to the action of cod liver oil locally or to its systemic action in mobilizing calcium and thus preventing tetany cannot be stated.

Cameron and Moorhouse (1925) demonstrated that in thyro-parathyroidectomized dogs, the diffusible calcium was lowered more than the non-diffusible; this, they believe, is the direct cause of parathyroid tetany. Liu (1927) demonstrated that cod liver oil increased both the diffusible and non-diffusible calcium in infantile tetany. That cod liver oil raises this fraction is further evidence that it may be more rational therapy for the treatment of parathyroidectomized dogs than the administration of large amounts of calcium.

SUMMARY

The addition of cod liver oil to a mixed stock diet for thyro-parathyroidectomized dogs delayed the onset of tetany and lessened its severity when it did occur.

Serum calcium in control and in test animals dropped to the tetanic level but tended to approximate the normal in 30 to 40 days in the animals given cod liver oil.

The point is further confirmed that low serum calcium is not always accompanied by tetany.

Cod liver oil relieves tetany and benefits parathyroidectomized dogs either by improving calcium absorption, mobilizing body calcium, or increasing the ratio of ionized to unionized calcium.

Thyro-parathyroidectomized animals have been carried through estrus, pregnancy and lactation by the addition of cod liver oil to the stock diet.

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THE EFFECTS OF HIGH TEMPERATURE ON THE HEART AND CIRCULATION IN INTACT ANIMALS

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The cardiovascular phenomena which result from a progressive elevation of body temperature have been observed in clinical cases of pyrexia and studied experimentally in man (for literature, cf. Bazett, 1924). The conclusion seems to be that, as the temperature increases, the heart accelerates, the skin vessels dilate, the arterial pressures tend to decline, the pulse becomes larger and often dicrotic, while the minute volume of the circulation alters very little, if at all. When the elevation of temperature has reached what may be termed the upper endurance level, death as a rule occurs from respiratory paralysis before significant changes in the heart or circulation have time to develop. Occasionally, when the heart is less resistant than normally, or the respiration more so, a circulatory crisis precedes the respiratory failure: the heart becomes extremely fast or irregular, its beat is feeble or alternating, and blood pressures fall to very low levels.

Previous experimental work. Experimental work has given us an insight into some of the mechanisms concerned in such a circulatory failure but a survey of literature shows that certain aspects of the subject require further study.

The reactions of the heart have been especially studied by perfusing the organ with nutrient solutions at different temperatures (for literature, cf. R. Tigerstedt, 1921). Most investigators agree that the heart rate becomes progressively faster until an optimum temperature, variously placed between 40° and 43°C., is reached. At higher temperatures, the rate declines rapidly until the heart stops entirely or the rhythm becomes irregular and the experiment terminates with ventricular fibrillation. The nature of the irregularity and the fundamental cardiac disturbances involved have not been studied electrographically. Observers (e.g., Bock, 1908; Knowlton and Starling, 1912) agree that the amplitude of contractions diminishes with an increase in temperature, at even the lower ranges, but a normal minute output is maintained through the increased rate. As temperatures exceed an optimum, the contractions diminish still fur-

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ther so that the minute output is no longer maintained. The alterations in the contraction process responsible for this cardiac depression are not yet fully understood. A considerable divergence of opinion also exists as to whether the circulatory effects of high temperatures are chiefly or solely due to a direct action on heart and blood vessels or whether they are partly induced by effects on the controlling medullary centers. Evidence on this question has been sought by heating the blood as it flows through the carotid arteries. The results reported are not concordant, however, probably because the effect of increasing temperature was not always limited to the cerebral circulation. Utilizing this method, Fick (1872) concluded that very considerable elevations of temperatures are without effect on the medullary centers controlling the heart and blood vessels. Cyon (1874) reported that a sudden great increase in temperature of the carotid blood caused a vagal slowing and a fall in blood pressure. Kahn (1904) described a dilatation of skin vessels and a moderate increase in blood pressure and heart rate as a result of sending warmed blood to the brain. Moorhouse (1911) found a dilatation of the limb vessels *enclosed* in a plethysmograph, an increased heart rate attributed to increased accelerator tone—preceded in occasional experiments by a preliminary slowing due to vagus stimulation. C. Heymans and Ladon (1925) were unable to establish any central effects of high temperatures in their cross-circulation experiments. It is apparent that certain essential information is still lacking for a satisfactory interpretation of the cardiovascular effects produced in hyperpyrexia. This is partly due to the fact that most of the investigations concerned themselves with special phases of the circulatory problem; and partly to the fact that the circulatory reactions in the intact animal are not necessarily the consequence of a high temperature alone. The experimental work of J. F. Heymans (1919), Haggard (1920), Flinn and Scott (1923), Barbour (1924), Adolph and Fulton (1924) has clearly shown that concurrent modifications in the physical and chemical characteristics of the blood take place which may have an effect on the circulation.

Methods of investigation. It was the purpose of the investigation reported in this paper to study such combined effects of high temperatures on intact animals by utilizing methods which have not hitherto been applied to the problem.

Two sets of experiments were carried out. In one, electrocardiograms and heart sounds were recorded simultaneously. By the former, the nature of the alterations in rate, rhythm and conduction were followed. The heart sounds were employed to study the changes in heart rate, cycle length and duration of systole as the temperature rose. The relation of systole to cycle length or the s/c ratio was especially studied in order to determine whether the duration of ventricular contraction deviated from normal values, established by Wiggers and Katz (for reference and discus-

sion, cf. Wiggers, 1923). The recent observations of Bazett and Sands (1926), it seems to us, bring forth additional evidence that important modifications in ventricular contraction are reflected in deviations from the normal duration of systole at comparable heart rates.

In another group of experiments, systolic and diastolic pressures and changes in the contour of the innominate pulse were recorded in addition to the heart sounds by means of optical manometers described by Wiggers and Baker (1924). Systolic and diastolic pressures were calculated by calibrating the optical manometers under static conditions. In addition to gaining information as to the state of the heart and circulation from contour changes and quantitative changes in pressures, these curves were utilized to calculate the changes in the isometric contraction and ejection phases. It may be noted that mean blood pressure was recorded coincidentally on a smoked drum in order to follow the general changes of pressure during the course of an experiment. In two experiments, samples of alveolar air and blood were also taken at different temperatures in order to study changes in the blood gases, alkaline reserve and pH.

In order to produce an elevation of temperature dogs were anesthetized with morphine and barbital and placed in a specially designed heating cabinet in which the lamps used for heating were so placed that direct sensory stimulation of the skin and possible actinic effects were not concerned.

Previous to placing the animal in the cabinet, the necessary minor operations were performed, the recording apparatus was attached, and a thermometer was inserted into the jugular vein, thus registering the temperature of the blood as it entered the heart. When placed in the cabinet, the dog's head and neck protruded from the box, making access to the carotid vessels, trachea and vagus nerves easy.

In some experiments of each set, the animals were cooled in a refrigerator for an hour previous to being placed in the heating cabinet. This was done in order to obtain a somewhat greater range of blood temperatures. The animals breathed naturally through a tracheal cannula to which a rubber tube, 12 inches in length, was attached, but in order to obtain the final cardiac effects without the complication of terminal asphyxia, artificial respiration was instituted toward the end of several experiments in which respiration suddenly failed.

Results. Eighteen experiments were performed; twelve were studied electrographically, and the rest by means of the optical central pulse. On tabulating and comparing the general results of all of these experiments, they were found to be so consistent in character, that they can be illustrated by means of three detailed charts (figs. 1, 2 and 3). The trend of the reactions thus plotted is obvious at a glance, but three features require brief comment: In order to study one aspect of the cardiac alterations obtaining during the progressive elevation of temperature, the actual dura-

tion of systole at different cycle lengths was compared with the calculated value of systole at corresponding cycle lengths. The latter was calculated according to the formula of Bazett and Sands (1926), $S = 0.25\sqrt{C}$, which appears to give the best correspondence with actual values obtained under normal conditions.

In order to study another change in the cardiac mechanism, the actual durations of the phases of systole and systolic ejection were plotted, as in figure 3. The vertical distance between any two points on these curves (i.e., the jet black area) gives an index of changes in the duration of the isometric contraction phase. In the same chart, the values for systolic and diastolic blood pressures are shown and the difference obviously gives the pulse pressure. The letters *A*, *B*, *C*, etc., appearing on the charts of figures 1 and 3 correspond to similar letters on records reproduced as figures 4 and 5, respectively.

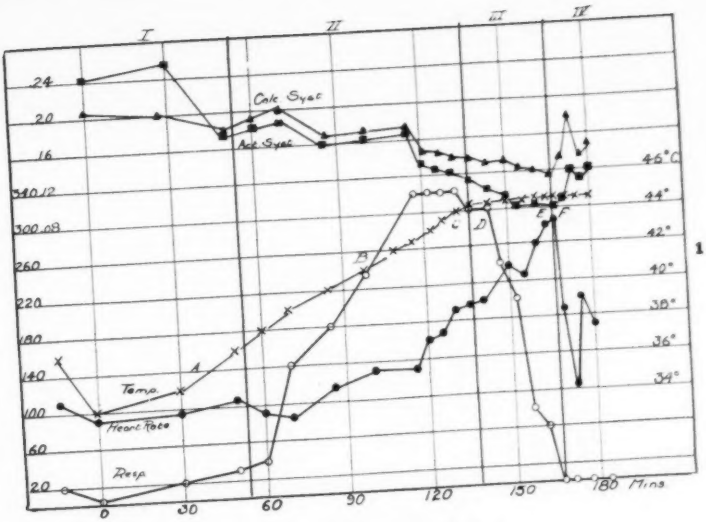
While the successive changes in the heart and circulation, as well as in the respiration, blood gases, etc. occur successively, it is desirable for purposes of description to divide the events during the progressive elevation of blood temperature into four stages: I, the recovery from previous cooling; II, the initial stage; III, the pre-critical stage, and IV, the critical stage. On the charts, these stages are demarcated by heavy vertical lines and the duration is indicated by time on the abscissae in figures 1 and 2. The lethal temperature varied with different dogs, but death always occurred when intracardiac temperatures ranged between 42° and 45°C. The time consumed in raising the temperature to the lethal point altered with experimental conditions, but was never less than 1 hour or longer than 3 hours.

Reactions during the stage of recovery from cooling (Stage I). When an anesthetized animal which had been previously cooled to 33° or 34°C. in a refrigerator was allowed to regain its normal temperature gradually, the cardio-respiratory reactions were affected comparatively little. As can easily be seen in the charts of figures 1, 2 and 3, the cardiac and respiratory rates increase slightly. Electrocardiographic records (fig. 5A) show no alteration in rhythm, in conduction, or in the form of the QRS complexes. Systolic and diastolic pressures (fig. 3) tend to fall somewhat and the durations of systole and systolic ejection decrease slightly. These changes are no greater, however, than can readily be accounted for by the increase in heart rate.

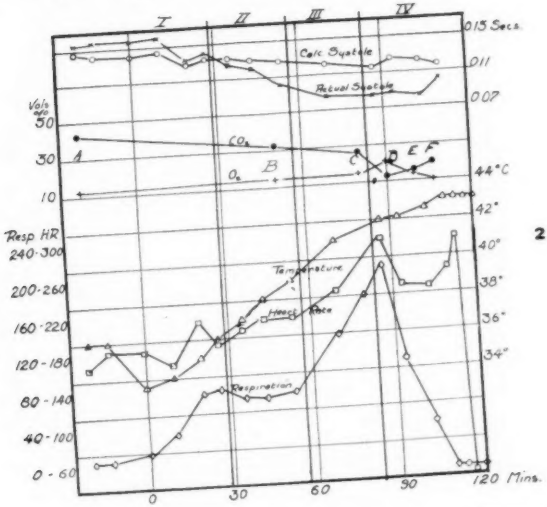
Fig. 1. Chart of consecutive changes during artificial rise of temperature in dog with intact vagus nerves. Letters A, B, C, etc., correspond to similar letters in figure 4. Discussion in text.

Fig. 2. Chart of consecutive changes during artificial elevation of temperature in dog with vagus nerves cut. Discussion in text.

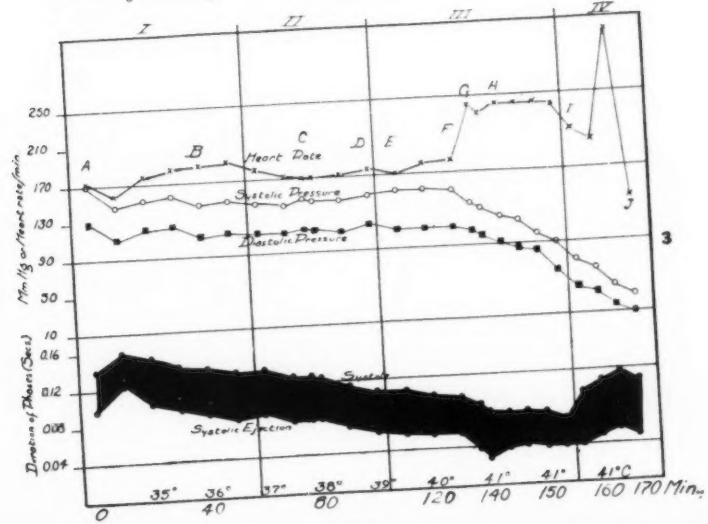
Fig. 3. Chart of successive changes during artificial increase in temperature of dog with vagus nerves cut. Letters A, B, C correspond to segments of records correspondingly lettered in figure 5. Discussion in text.


I
II
III
IV
Calc Systole
Act. Systole
Temp.
Heart Rate
Resp.
A
B
C
D
E
F
46°C
44°
42°
40°
38°
36°
34°
180 Mins.

1


I
II
III
IV
Calc Systole
Actual Systole
CO2
O2
A
B
C
D
E
F
Temperature
Heart Rate
Respiration
0.13 Secs.
0.11
0.09
44°C
42°
40°
38°
36°
34°
120 Mins.

2


I
II
III
IV
A
B
C
D
E
F
G
H
I
J
Heart Rate
Systolic Pressure
Diastolic Pressure
Systole
Systolic Ejection
35°
36°
37°
38°
39°
40°
41°
41°
41°C
170 Mins.

3

When calculated and actual systole are compared as to lengths (figs. 1 and 2), the former is found to be much shorter than the latter as long as the body temperature is low, but as soon as it approximates the normal (37°C.), they become very nearly identical. Since this occurred, regardless of whether the vagus nerves were intact or cut (cf. figs. 1 and 2) and since the blood gases remained unaltered, this appears to be a direct effect of low temperature on the heart. A comparison of the pressure curves obtained during this stage (segs. A–B, fig. 4) shows no contour variations which indicate significant alterations in the dynamics of the heart beat, or in the peripheral resistance.

Changes in the initial stage (*Stage II*). The initial stage begins at the point where the blood temperature has reached its normal value again (usually 37°C.), and is arbitrarily considered to pass into the pre-critical stage as soon as evidence of cardiac abnormality is recognized, regardless of the temperature at which such changes occur.

The respiratory and cardiac changes during this phase are shown in figures 1, 2 and 3. When the vagi are intact (fig. 1) respiration increases both in rate and depth, becoming rather forcible towards the end of this period. When the vagus nerves are cut, however, (fig. 2) the increase in respiratory rate is not so marked and may not reach its maximum during this phase. The heart rate increases progressively reaching 180 to 220 per minute toward the end of this period. Before the heart begins to accelerate, however, a preliminary decline in rate is quite constantly noted. As the acceleration occurs both in animals with their vagus nerves intact and those with vagus nerves severed—the change being merely a matter of degree—this phenomenon cannot be due to a vagal mechanism. It may, however, as Moorhouse suggested, be due to increased accelerator activity. If this is the case we should expect to find a greater shortening of systole than would otherwise occur, for Wiggers and Katz (1920) have shown that the s/c ratio is much below the calculated value, in such a case. A comparison of the calculated and actual systolic lengths indicates that this in fact occurs. There is a gradual tendency throughout the increasing temperature for actual systole to abbreviate more than that calculated. The difference is not quite so great when the vagus nerves are intact (fig. 1) as when they are severed (fig. 2). In the latter case, the difference has a value of 0.023 second at the end of this initial phase. These results therefore give additional evidence that the accelerator mechanism is active during this stage of temperature elevation During this acceleration the S-A node remains the pacemaker, as shown in segments B and C of figure 5. An analysis of these electrocardiograms in lead II reveals three important changes, viz., 1, a slight abbreviation of the P-R interval (0.120 sec. to 0.108 sec.) indicating a facilitation of conduction as the temperature rises; 2, a gradual decrease in the amplitude of the QRS complexes with a splitting of their

summits, and 3, a gradual reversal of a negative T wave to a positive form. These changes, however, do not warrant the conclusion that the heart is in any way deleteriously affected.

A glance at the chart of figure 3 and a comparison of segments B and C of figure 4 show that systolic and diastolic pressures undergo no significant changes, in fact diastolic pressure rises slightly. The contour of the pressure curves gradually alters, however. Thus, in segment C of figure 4 the ascending limb is more gradual, the primary vibration is reduced to a momentary anacrotic halt and the curve rises slowly to a rounded summit. With the end of ejection, the incisura becomes deeper and the after-vibrations are augmented. The rounded contour of the curve indicates that the vigor of ejection is diminished. At the same time, the duration of systole

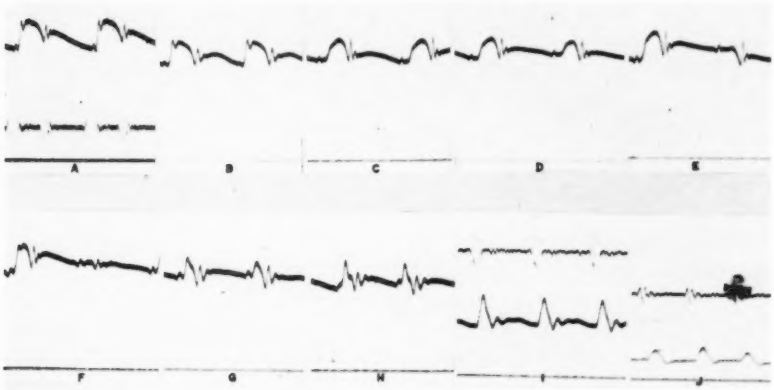


Fig. 4. Records of innominate arterial pulse recorded in relation to base line. Segments lettered to correspond with letters on chart of figure 1. Segments A, I, J contain in addition heart sound records. Discussion in text.

and its phase of ejection become shorter. These facts, taken in conjunction with a decreasing pulse pressure, indicate that the volume of systolic discharge is diminishing. The increase in diastolic pressure, on the other hand, is evidence that the acceleration of the heart is still able to compensate for the reduced systolic discharge. Blood-gas analyses during this stage showed that the CO_2 content was only slightly diminished but the O_2 content was slightly increased. We may, therefore, conclude that the initial changes in the heart—i.e., the acceleration, the excessive abbreviation of systole, the reduction of the A-V conduction time, the decreasing velocity of ejection, etc.—are effected by changes in temperature and not by alterations in the chemical constitution of the blood.

The precritical stage (*Stage III*). This stage extends from the time when

evidence of cardiac involvement first begins to appear to the time when circulatory failure has proceeded to such a point that conditions become critical. The changes which occur during this stage of pyrexia are no longer the sole effect of the increasing temperature, for, as illustrated in figure 2, decided changes occur in the blood itself. At the end of this stage, D, alveolar CO_2 and blood CO_2 decrease markedly, and pH readings indicate that this is accompanied by a condition of slight alkalosis. On the other hand, the O_2 content increases as a result of deeper breathing. It is obvious that the effects of acapnia on the heart and circulation can no longer be ignored.

A comparison of the charts in figures 1 and 2 shows interesting differences in respiratory rate during this stage. When the vagi are intact the rate (fig. 1) of respiration rapidly decreases and as in this instance may actually fail. When the vagus nerves are cut, however, the respiratory rate continues to increase during this phase or, more accurately expressed, the great increase in respiratory rate is retarded, and the ultimate reversal to slowing and respiratory failure is delayed. These results suggest that early respiratory failure is partly contingent upon the integrity of the vagus nerves, and not entirely due to the increasing acapnia and alkalosis. This accords also with the observations of Porter and Newburgh (1917) that exhaustion of the respiratory center in pneumonia can be prevented or delayed when the path of afferent impulses from the lungs is severed by cutting the vagi.

The obvious circulatory changes during this phase are the extreme acceleration of the heart (figs. 1, 2 and 3)—sometimes mounting to rates exceeding 300 per minute—and the progressive decline of both systolic and diastolic pressures (fig. 3). A study of the electrocardiogram (segments D and E, of fig. 5), shows that the rapid rhythm is still controlled by the S-A node, and calculations show that the conduction time, as represented by the P-R interval, is considerably less than normal (0.120 sec. to 0.074 sec.). The duration of systole also falls more and more below its calculated value as is clearly shown by the greater divergence of the lines representing these values in figures 1 and 2. One is inclined to attribute this to an increasing accelerator activity, but such an interpretation may not be made until we have further evidence as to the effect of high temperatures on the heart itself, and know more about other dynamic changes (e.g., venous pressure changes).

The association of a rapidly declining blood pressure and a very rapid heart is a symptom-complex which naturally suggests that a condition similar to that found in surgical or traumatic shock may be developing, i.e., that the fall of blood pressure is due to peripheral causes or to changes in the volume of circulating blood, while the cardiac acceleration is secondary. It is precarious, however, to draw such a conclusion without a more care-

ful study of the circulatory dynamics, and final conclusions on this point cannot be reached on the basis of experiments completed in this research.

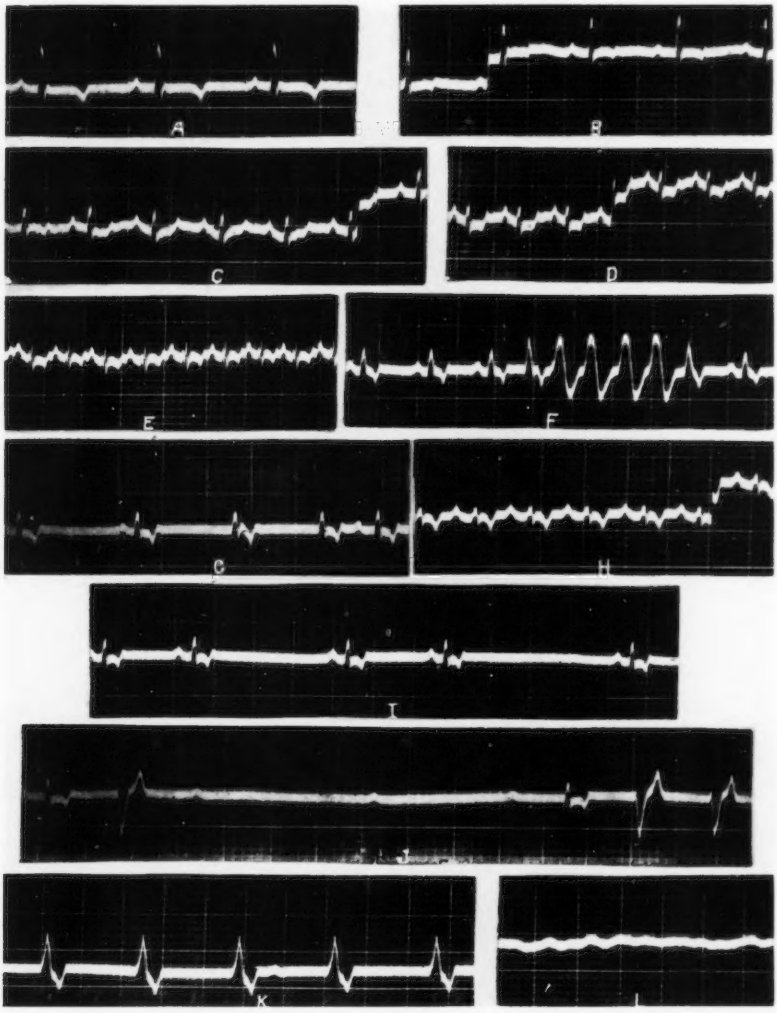


Fig. 5. Electrocardiograms (lead II) taken at various stages of pyrexia. Segments lettered to correspond with letters on chart of figure 3. Discussion in text.

A suggestion is contained, however, in the contour changes of the arterial pulses (fig. 4, segments F, G and H) taken during the period of declining

blood pressure. Their peaked appearance and rapid drop during the latter portion of systole indicate that a low total peripheral resistance undoubtedly exists. Furthermore, the progressive and rapid decrease in the duration of systole and its ejection phase, which are far below their calculated values, can be harmonized easily with the conception that the return of venous blood and the initial intraventricular tension are decreasing. But at all events, the heart itself does not escape the combined influence of a high temperature and changes in the blood. For example, a comparison of segments D, E, F and G in figure 4, shows that alternation develops and progresses to a degree at which only every second systole is effective in the ejection of blood (Segment F.) There is little reason, however, to attribute the circulatory failure to this condition, for even when alternation becomes extreme, the blood pressure and pulse pressure seem to be maintained fairly well, and curiously enough, as the pressures fall abruptly, the alternation becomes less in degree or disappears (cf. fig. 4, segments F, G, and H).

Changes during the critical stage (*Stage IV*). The critical stage which follows and continues until death is characterized by the progressive depression of respiration, by an abrupt slowing of the heart and by the development of a variety of cardiac irregularities. The respiratory failure always precedes the complete stoppage of the heart and is the cause of death, but the marked irregularity of the heart often develops an appreciable interval before respiratory failure occurs, especially when the vagus nerves are cut. Some of the typical cardiac rhythms developed during this stage are shown in figure 5, segments F to L. Segment F shows a short paroxysm of ventricular tachycardia (330 per min.), which after a single nodal beat returns abruptly to its sinus rhythm (205 per min.). Segment G shows a pronounced slowing of the S-A rhythm with an occasional transfer of the pacemaker to the A-V node. In segment H, taken a little later, a return to a rapid form of sinus rhythm is shown, similar to that of segment E, except that the P-R interval now begins to lengthen again and the T wave is inverted. In segment I, a dominant slow sinus rhythm is displayed but the beat is obviously very irregular. Segment J shows the stage in which complete A-V block is developed, and an idio-ventricular rhythm maintains the beat of the heart. In segment K, the full development of this ventricular rhythm is shown, and evidence of auricular activity now appears only occasionally. Segment L shows the terminal ventricular fibrillation shortly after its development. The inferences that may be drawn from a consideration of these records are briefly: a, that the S-A node is very resistant to the effects of high temperature and concomitant changes in the blood such as result in hyperpyrexia and remains the pace maker until very near to the end; b, that high temperature first increases the A-V conduction rate and later produces an A-V block; c, that the intra-

ventricular centers dominating the rhythm of the heart towards the end appear to be somewhat more resistant to the effects of high temperature and concomitant changes in the blood, than the supraventricular nodes.

The dynamic changes observed during the critical stage are illustrated in figure 3. Systolic and diastolic pressures fall very rapidly, and the pulse pressure becomes extremely small. The latter is also shown in the optical records of segment I, figure 4. It displays a very simple contour. The curve rises to a sharp peak and falls equally rapidly during the latter portion of systole. Its amplitude decreases progressively until the end. It may also be noted incidentally that blood pressures are now so low that only a single heart sound is recorded.

Deleterious changes in the contractile mechanism are evidenced by further changes in the duration of systole and its phases. Instead of continuing to decrease in length, as has been the general tendency previously, both systole and systolic ejection now show a pronounced increase in duration (fig. 3). In spite of the low arterial pressure, the isometric contraction phase is also greatly prolonged.

Analysis of the dynamic changes in this terminal phase clearly shows that cessation of the circulation is precipitated by the direct changes in the heart. These develop regardless of whether terminal asphyxia supervenes or whether this is prevented by the institution of artificial respiration.

SUMMARY

The successive changes which take place in the heart and circulation when the blood temperature of an anesthetized dog is first lowered and then gradually raised to a lethal point were studied.

The course of events can be divided into four stages, during which the following essential changes take place:

Stage I. The interval of recovery from previous cooling. The heart rate increases progressively; systolic and diastolic pressures fall slightly. The only significant observation during this interval is that cooling reduces the duration of systole much more than can be accounted for by an effect of slowing alone. As blood temperature returns from 33° or 34°C. to 37° or 38°C. this disparity disappears.

Stage II. The initial stage extends to the point where cardiac abnormalities are first recognized. Respiration and heart rate increase progressively; the S-A node remains the pacemaker even when the rate reached is 180 to 220 per minute. The *P-R* interval is slightly abbreviated, the QRS complex is smaller and often bifurcated, and the negative T wave changes to a positive form. The arterial pressure curves indicate that systole and the velocity of ejection are reduced. The probability that all of the effects are attributable to high temperature is discussed.

Stage III. The precritical stage extends to the time when circulatory con-

ditions become critical. Alveolar and blood CO_2 are decreased, and a condition of mild alkalosis exists. The S-A node still controls the rhythm and A-V conduction time is further shortened. The heart accelerates extremely; systolic and diastolic pressures decline progressively. The arterial pressure curves develop a peaked contour and fall rapidly during the latter portion of systole, suggesting a low peripheral resistance. Durations of systole and systolic ejection are reduced more than can be accounted for by the rapid rate. Alternation frequently develops but may disappear again as the temperature increases further.

Stage IV. During the *critical stage*, marked irregularity of the heart occurs before respiration ceases. Electrocardiographic studies show a variety of rhythms which lead to the conclusion a, that the S-A node is very resistant to effects of high temperature and remains the pacemaker until very near the end; b, that ultimately A-V block and various types of ventricular rhythm develop. Systolic and diastolic pressures fall rapidly and the pulse pressure is small. The duration of systole and its phase suddenly increases in length as the heart finally fails. The cessation of the circulation is precipitated by the direct changes in the heart, regardless of whether terminal asphyxia supervenes or whether this is prevented by the institution of artificial respiration.

In conclusion, the writer wishes to express his gratitude to the staff of the Department of Physiology for their coöperation, and especially to Prof. C. J. Wiggers, who suggested this investigation and helped in interpreting the results.

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HISTOLOGICAL STUDY OF THE THYROID OF THE GUINEA PIG IN EXPERIMENTAL SCURVY

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The effect of diet on the endocrine glands has been but little investigated. Bensley (1) has shown the thyroid of the opossum to undergo hyperplasia during chronic inanition. Jackson (2) has shown decrease in size of the gland and an increase in interfollicular tissue in white rats during inanition. Lindsay and Medes (4) have reported retrogressive changes in the testes of the guinea pig in scurvy. These authors state the changes to be the same as in chronic inanition. McCarrison (3) has reported changes in the thyroid as a result of vitamin A deficiency, but gives no conclusive findings.

The thyroid is definitely related to iodine metabolism. Vitamins play an important rôle in mineral metabolism in general. The following investigation was undertaken to determine whether the histological picture of the thyroid is changed by a diet lacking in or free from vitamin C. Chronic scurvy and acute scurvy as compared to normal controls and to starvation were studied.

METHOD. Guinea pigs were fed on a basal diet consisting of:

Alfalfa meal	50 per cent
Wheat flour	50 per cent
Oats, Water	ad lib.

supplemented by orange juice administered daily by means of a pipette.

The starvation diet consisted of water and 1 cc. of orange juice daily.

Animals of as nearly the same age and weight as possible were selected for the experiments, and the sexes divided equally between control and experimental groups.

The animals were kept on diet until the disease was in its most advanced stage, then killed by decapitation. The thyroid was immediately dissected out intact with the trachea, and placed in Zenker's fluid for fixation. The tissue was imbedded in paraffin and sectioned six to eight microns thick. Serial sections of the glands were made and stained with Delafield's hematoxylin and eosin.

The slides were studied as to *1*, condition of follicles; *2*, colloid; *3*, epithelium, and *4*, interfollicular cells. Slides from the same areas of the thyroid were used in comparison. (Table 1, figs. 1 and 2.)

Discussion. A histological study of the thyroid revealed in the case of chronic scurvy a marked decrease of colloid, an increase in interfollicular cells, a general lengthening of the cells lining the follicles, and an increase in the number of vacuoles in the colloid. The group which was on experiment for 97 days evidenced changes much more marked than those on diet for 51 days. The acute scurvy group revealed changes similar to those in

TABLE 1

Results

	Number of animals	Days on experiment	Microscopic findings			
			Follicles	Colloid	Epithelium	Interfollicular cells
Control animals	9	8–97	Rounded, regular, numerous	Uniformly stained, few vacuoles	Cuboidal	Few
Chronic scurvy	6	51–97	Irregular, enlarged, fewer in number	Totally absorbed from many follicles Large vacuoles	Elongated	Markedly increased
Acute scurvy	2	30–33	Irregular	Decreased in amount, more vacuoles	Elongated	Slightly increased
Starvation	2	8–10	Same as control	Same as control	Same as control	Same as control

chronic scurvy but less marked. The thyroids of the animals on starvation diet showed no changes that could be noted by the methods of study employed.

Other writers have noted changes in the thyroid and other glands of internal secretion during chronic inanition. Our studies were of acute inanition rather than chronic. The thyroid from animals starved for eight to ten days showed no differences from the normal.

The decrease in the amount of colloid could be accounted for by Bensley's hypothesis (4). He assumes resorption of colloid only under conditions in which the normal direct secretory activity of the thyroid is insufficient

to meet the functional demands of the body. The resorption of the colloid in these experiments may mean inability to meet the functional needs of the body or a depression of thyroid activity below the normal.

The increase of interfollicular cells may be due to the fact that the colloid of some of the follicles is wholly absorbed, and the cells fill in the spaces, or to a proliferation of the cells lining the follicles. However, few mitotic figures or dividing cells were observed (fig. 2*m*). The lengthening of the cells lining the follicles might be due to decreased pressure within the follicle, resorption of the colloid allowing the cells to lengthen out.

At the time of the termination of the experiments, the animals were in a very much debilitated state being so weakened that death seemed evident within 24 to 48 hours.

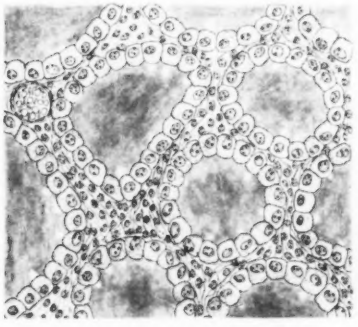

Fig. 1

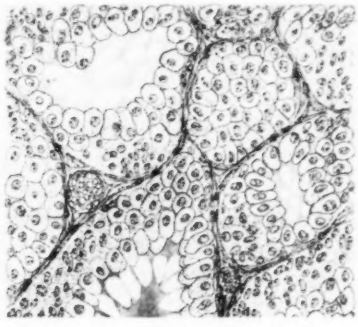

Fig. 2

Fig. 1. Drawing of typical area of thyroid from normal guinea pig. × 500.

Fig. 2. Drawing of an area of thyroid from the same region of the gland as above but from guinea pig 97 days on scorbutic diet. × 500.

The duration of the experiment had a marked effect on the condition of the thyroid. Acute scurvy (30 to 33 days) revealed changes which were similar to those noted in chronic scurvy, but less marked. Starvation caused no changes.

Vitamin D has long been known to be a factor in calcium metabolism. Simmonds, Becker and McCollum (5) have linked vitamin E with iron metabolism. As the thyroid is known to be associated with iodine metabolism and vitamin C deficiency produces changes in the thyroid as noted above, the results of this investigation might be interpreted as an indication that vitamin C functions in regulating iodine metabolism. The changes in the thyroid may be due however to the general debilitating effect of scurvy which affects all parts of the body.

CONCLUSIONS

1. As a result of scurvy the thyroid gland shows an increased amount of interfollicular cells, a decrease in colloid, an increase in the number of vacuoles, and a lengthening of the cells lining the follicles, the degree of change depending on the length of time the animals survive the scurvy-producing diet.

2. Acute starvation of guinea pigs reveals no changes in the thyroid by the present method.

3. The results are interpreted as an indication of pathology in the thyroid as a consequence of vitamin C deficiency.

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PENETRATION OF ULTRA-VIOLET RAYS THROUGH CLOTHING MATERIALS

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A series of experiments designed to shed some light on the variability of clothing materials to transmit the actinic rays of the sun has been in progress at the Utah Agricultural Experiment Station since January 1, 1926, where the problem is of more than general interest. The station is situated at an altitude of 4700 feet, one thousand miles inland where fogs seldom occur, and being away from manufacturing plants which might pollute the air with smoke the problem becomes one of peculiar regional interest.

A preliminary experiment during the summer months which made use of the direct rays from the sun to activate food materials normally not anti-rachitic was suggestive of several points of attack. In order that work might be carried out under controlled conditions throughout the winter months, a rich and constant source of ultra-violet light was thought desirable; accordingly, the laboratory was equipped with a Hanovia A. C., air-cooled quartz mercury vapor lamp run at 220 volts and 2.5 amperes.

PART I. During the progress of this initial experimental work, a search of the literature led early to the conviction that information on several of the various factors involved would be necessary before a standardized procedure could be intelligently adopted. The purpose of Part I is to report some of these findings which are of sufficient general interest to warrant presentation and at the same time to state methods of procedure which, unless later statements are made to the contrary, will form the basis of subsequent experimental work designed to answer specific questions relating to the problem in mind.

McCollum's "line test" (1925) for rickets was accepted as the best indicator available for the detection of the anti-rachitic potency of a normally inert food substance activated by treatment with ultra-violet rays; cottonseed oil was selected as the inert substance lending itself most

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easily to such activation. All oil was exposed to the light source at a distance of 12 inches in 44-gram quantities spread out in porcelain plates 8 inches in diameter. The lamp was allowed to run 5 minutes before each quantity of oil was exposed, because, according to Luckiesh (1927), the intensity of the light increases to a constant after an initial period of heating. All rats used were from animals of healthy stock originally secured from the Household Science Departments of the University of California and Mills College, respectively. When approximately 30 days old the rats were put on McCollum's rickets-producing diet 3143 (McCollum and Simmonds, 1925); at the end of 3 weeks they invariably gave external indication of rickets by a peculiar flat-footed shambling gait. The animals otherwise remained healthy in appearance and were well behaved. From the time they were 3 weeks old until they were sacrificed for examination they lived in wire cages adequate in size to accommodate comfortably one male and one female. The cages were bedded with pine shavings, screened with newspapers to exclude the light, and kept on shelves in a well-ventilated fourth-floor west room. The windows of this room were furnished with shades.

At the expiration of the preparatory period of 3 weeks on the rachitic diet, never fewer than two controls were etherized, and the distal ends of the tibiae and femora invariably gave an unequivocal line test for rickets. Test diets designed for the rachitic animals were always prepared in quantities sufficient to last the remainder of the experimental period and were kept on ice. The animals were given fresh water and the ration *ad libitum* daily. Particular attention was given to the freshness, cleanliness, and quantity of their daily supply of food because of the warning that fasting has a beneficial effect on rickets (McCollum *et al.*, 1922).

Experimental. 1. *Optimum time of irradiating cottonseed oil with a mercury vapor quartz lamp.* It is a well-established fact that inert oils, such as cottonseed, can be made anti-rachitically potent by exposure to ultra-violet radiation. However, there exists a difference of opinion among investigators as to the optimum time of irradiation. Hess (1924) found that cottonseed oil when rayed for one hour at a distance of one foot acquired anti-rachitic properties. Steenbock and co-workers (1925) report the optimum time of irradiation of olive and cod liver oils to produce maximum anti-rachitic potency to be from 30 minutes to 5 hours. Hess and Weinstock (1925) later report that "vegetable oil can be activated by an exposure to the mercury vapor quartz lamp for a period of 2 minutes or less." The purpose of the work reported herewith was to obtain data relative to the optimum time of irradiation of cottonseed oil when used in quantities making up 15 per cent of experimental diets. This is the usual amount of fat allowed in basal diets, and it was planned to use this amount in further work. One quantity of oil was exposed 10 minutes, another 5 minutes, and another 2 minutes.

Three groups, each composed of four rachitic rats (2 males and 2 females), received the diets. At the end of 7 days the animals were etherized and examined, as had been done with controls. All the tibial bones showed evidence of healing. However, the tibial bones of the animals in group I, fed the oil irradiated for 10 minutes, did not have rachitic metaphyses. The proliferative zone of cartilage was narrow. The osteoid cells were arranged in a definite well-defined pattern. There were extensive calcium deposits, as indicated by the density of the blackened area on both the diaphseal and epiphyseal sides of the cartilage. The bones of the animals in group II, fed the oil which had been irradiated 5 minutes, so far as could be observed presented identical pictures. The bones of the rats in group III, which had been fed oil irradiated for 2 minutes, revealed some healing of the rachitic metaphyses, but the proliferative zones of cartilage, although regular, were much wider than the proliferative zones of cartilage in the bones of groups I and II. The calcium deposits were irregular and not as dense. The osteoid cells were not clearly defined; or, in other words, the cellular organization was not as regular as it appeared to be in the bones of groups I and II.

2. *A comparative study of the anti-rachitic potency of irradiated cottonseed oil and cod liver oil.* Cod liver oil is the well-known specific for rickets. McCollum (1922) reports a positive line test at the end of the fifth day after the addition of 2 per cent cod liver oil to diet 3143. The purpose of the present study was to compare the healing effects brought about by 15 per cent irradiated cottonseed oil with the cure induced by the addition of 3 per cent cod liver oil to a rickets-producing diet. Twenty rachitic animals were divided into two groups. Group 1a–1e received the rachitic diet plus 15 per cent cottonseed oil which had been irradiated for 5 minutes. Group 2a–2e received the rachitic diet plus 3 per cent cod liver oil and 12 per cent non-irradiated cottonseed oil added to make a total of 15 per cent fat. Two animals from each group were etherized and the tibiae and femora subjected to the line test each two days until 10 days had elapsed.

The results obtained are shown clearly in the accompanying reproductions of photomicrographs. The bones all gave a positive line test on the sixth day. At the end of 8 days the rachitic metaphyses had disappeared; the proliferative zones of cartilage were narrow; the osteoid cells were arranged in a definite well-defined pattern. By the end of the tenth day the bones were not distinguishable from the bones of normal rats.

3. *Curative effects of smaller amounts of irradiated cottonseed oil.* The results of a piece of experimental work completed in this laboratory by two graduate students,[3] under the supervision of the senior author, are of interest in their bearing upon the problems in mind. A study was made of

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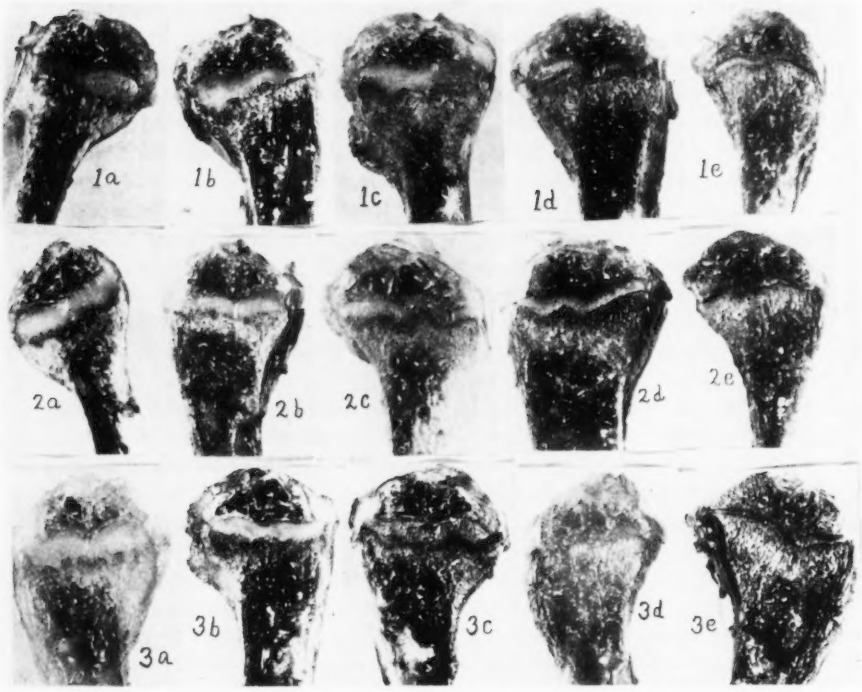
the anti-rachitic and growth-promoting potencies of the rachitic diet and 10 per cent of oil. Three rachitic animals were included in each group. Group I received the diet with all the oil irradiated for 5 minutes; two-



Figs. 1a–1e. A series of photomicrographs of the tibiae of rats which received the rickets-producing diet 3143 plus 15 per cent irradiated cottonseed oil. The photomicrographs were taken at intervals of two days, as indicated by the subscripts. Note the progressive disappearance of the rachitic metaphyses.

Figs. 2a–2e. A series of photomicrographs from rats that received the rickets-producing diet 3143 plus 3 per cent cod liver oil. Note the corresponding disappearance of the rachitic metaphyses (see figs. 1a–1e).

Figs. 3a–3e. A series of photomicrographs showing the relative potency of the oil irradiated through different clothing filters. 3a, photograph of tibia of rat which received the rickets-producing diet plus 15 per cent cottonseed oil which had been irradiated through the pongee filter; 3b, through baby flannel (wool) filter; 3c, through crepe de chine (silk) filter; 3d, through meadow lane (cotton) filter; and 3e, through artificial silk filter.

fifths of the oil in the diet of group II was similarly irradiated, while but one-fifth of the oil from group III was irradiated. The rats were weighed

individually each week. An interesting detail which the figures revealed is the fact that at the end of the experimental period every animal weighed more than the average weight of normal rats, as given by Donaldson (1924).

The results of this experiment may be summarized as follows: The tibial bones of the rats which received 10 per cent of irradiated oil showed a deposition of lime salts in the proliferative zone of cartilage which compared well with that found in the tibial bones of normal rats. The cartilaginous area of the bones of group II was wider than normal and the zone of calcification appeared irregular. The bones of group III showed an irregular cartilaginous zone at least twice the normal width, and the zone of calcification appeared irregular. The amount of calcium which had been deposited, however, suggested that the healing process had started.

CONCLUSIONS

McCollum's line test for rickets shows that cottonseed oil irradiated for five minutes brings about healing of the rachitic metaphyses to the same degree as oil irradiated for ten minutes, when fed in amounts making up 15 per cent of rachitic diet 3143. The healing brought about by oil irradiated for two minutes was distinctly less.

It was found that 15 per cent of cottonseed oil irradiated for five minutes is as efficacious as 3 per cent of cod liver oil in the healing of experimental rickets under the conditions described. Since a rickets-producing diet fortified by 10 per cent of irradiated oil promoted growth and cured rickets in an equally efficacious manner, it would seem that 15 per cent of oil in the diet is a liberal amount.

PART II.[4] Ultra-violet radiations have limited penetrative power since they are readily absorbed by atmospheric gases, moisture, smoke and ordinary window glass. Clothing must also be regarded as a filter which may screen out some of the effective radiations.

Experimental. Materials of approximately the same weight were chosen. All the materials were white or nearly white. A description of the fabrics used is given in table 1.

The interspace between fibers was determined by means of a microscopic micrometer. However, this is only an approximate determination as the ends of the fibers protruding into the interspace lessened the space, and this reduction could not be measured. The reduction in the wool material was appreciable since the wool fiber was especially "hairy."

Filters of meadow lane, baby flannel, crepe de chine, pongee and artificial silk were interposed between cottonseed oil and the mercury vapor quartz lamp. The oil was otherwise irradiated under the conditions described in Part I. Twenty rachitic rats, 29 to 31 days old, were divided

[4] The major part of the work under Part II has been under the direction of the junior author.

into five groups. Each group was given a diet consisting of 85 per cent of diet 3143 and 15 per cent of cottonseed oil which had been irradiated through a clothing material filter. The McCollum line test was made at the end of eight days, the results of which are shown in the accompanying reproductions of photomicrographs from a typical tibial bone from one animal from each group.

Microscopic examinations showed that the tibial bones from the animals which had received the oil irradiated through artificial silk filter did not have rachitic metaphyses. They were indistinguishable from normal bones. The bones from the animals which had received the oil irradiated through meadow lane (cotton) filter showed narrow proliferative zones of cartilage, indicating a marked degree of healing. The cellular organization

TABLE 1

Descriptions of materials used

COMMERCIAL NAME OF FABRIC	FIBER	WIDTH	COST PER YARD	COLOR	NUMBER OF THREADS PER CENTIMETER	WEIGHT OF 4 x 4-INCH PIECE	INTERSPACE BETWEEN 2 THREADS	PER CENT OF ASH
		inches				*grams*	*mm.*	
Baby flannel.....	Wool	25	$1.25	White	15	1.486	0.0333	0.267
Crepe de chine.....	Silk	40	$3.75	White	30	1.046	0.17	35.23
Pongee.....	Wild silk	32	$1.25	Natural	26	0.493	0.074	0.944
Meadow lane.....	Cotton	32	$ .49	White	30	1.005	0.126	0.2377
Viscose artificial silk.....	Cellulose	*	*	Pink	42	1.348	0.0778	0.3416

* Artificial silk obtained from American Rayon Products Corporation; cost and width not given.

of these bones was regular. The bones from animals which had received oil irradiated through baby flannel (wool) filter, or pongee filter, or crepe de chine (silk) filter showed no evidences of healing. The rachitic metaphyses were wide; the cellular organization was disrupted by masses of cartilage; there were no provisional zones of calcification. In short, the bones gave a clear negative line test.

CONCLUSIONS

Under the experimental conditions described, the baby flannel, pongee and crepe de chine filter out the ultra-violet radiations which are anti-rachitically potent. The small amount of interspace in the baby flannel and the large percentage of ash in the crepe de chine and the pongee may have influenced their non-transmissibility.

The artificial silk and the meadow lane materials transmit the ultra-violet radiations which are effective in healing rickets. However, these materials have the largest interspace and the smallest percentage of ash. These facts seem to indicate that there are factors other than the fiber which may influence the transmissibility of ultra-violet radiation through clothing material.

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THE EFFECT OF LOW GLYCOGEN CONTENT ON THE FATIGUE CURVE AND ON LACTIC ACID FORMATION IN EXCISED MUSCLE

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In a paper by Olmsted and Harvey published in 1926, it was concluded that frogs' muscles in which no glycogen could be detected after prolonged insulin convulsions, could be made, upon stimulation in intact animals, to contract for a considerable length of time.

It might be argued that glycogen might be formed in a sufficient quantity from the liver, to supply the necessary energy. The experiments reported in the present paper were designed to meet this objection, being a repetition of the above work with the substitution of isolated muscles for those in the intact animal. At the same time, the muscles were analysed for lactic acid, free carbohydrate and elemental phosphorus.

METHOD. In order to make sure of having a sufficient quantity of material, large bull frogs (Rana catesbeiana) weighing 250 grams were used. The weight of each portion of gastrocnemius used averaged 1.5 gram; and of each portion of thigh muscle, 2.0 to 4.0 grams. The frogs were injected with 15 or 20 units of insulin. In the succeeding twenty-four to ninety-six hours the animals passed through a series of convulsions, and were also stimulated in the manner described by Olmsted and Harvey, to cause depletion of glycogen stores.

One gastrocnemius and one thigh (divided into inner and outer halves) were used as controls in each case, being placed in the reagents immediately after the frog had been killed. The opposite gastrocnemius was isolated and stimulated to fatigue by means of inductorium shocks, while the thigh muscles (divided as above) were exposed to warm chloroform vapour until rigor became marked.

For analysis each muscle—or portion of muscle—was cut in half, one half being placed immediately in hot potassium hydroxide solution, to be analysed for glycogen, the other half in ice cold hydrochloric acid for determinations of lactic acid, free carbohydrate and phosphorus. The Pflüger method (1903) was used for determining glycogen. The solution used for calculating the glycogen content was diluted to between 10 and 25 cc. only, to avoid the errors contingent upon using so weak a solution as would have

resulted from a dilution to 100 cc. Bunsen valves were fitted to the rubber-stoppered flasks used in Shaffer-Hartman (1921) method of estimating the sugar content of the glycogen solution. The Hirsch-Kauffman (1924)

TABLE 1

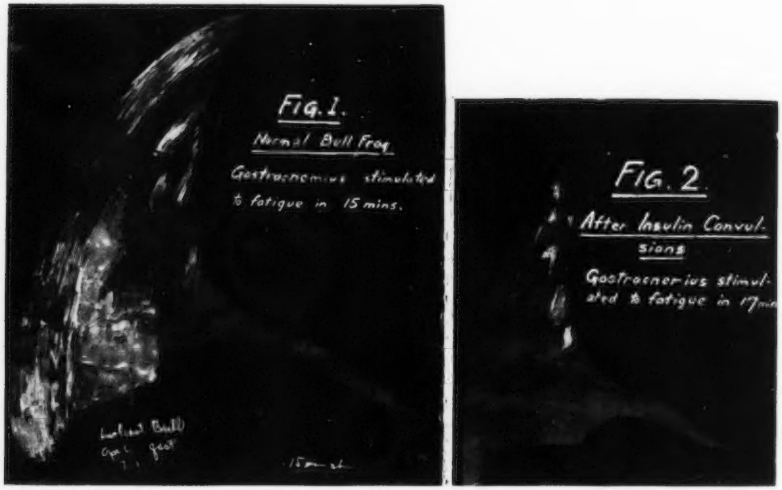
Normal bull frogs

FROG		GLYCOGEN	LACTIC ACID	FREE SUGAR	PHOSPHORUS
Concentration of substances in gastrocnemii before and after electrical stimulation to fatigue					
No. 52	At rest	0.36	0.09	0.07	0.11
	Fatigued	0.15	0.34	0.08	0.11
No. 54b	At rest	0.38	0.06	0.05	0.11
	Fatigued	0.14	0.30	0.07	0.11
No. 54c	At rest	0.38	0.12	0.06	0.11
	Fatigued	0.20	0.27	0.09	0.11
Average	At rest	0.37	0.09	0.06	0.11
	Fatigued	0.16	0.30	0.08	0.11
	Difference	−0.21	+0.21	+0.02	±0
Concentration of substances in thigh muscles before and after chloroform rigor					
No. 52	Normal	0.19	0.09	0.05	0.10
	In rigor	0.02	0.39	0.06	0.14
	Normal	0.30	0.11	0.07	0.10
	In rigor	0.04	0.22	0.07	0.15
No. 54b	Normal	0.29	0.08	0.06	0.11
	In rigor	0.10	0.33	0.07	0.14
	Normal	0.20	0.11	0.06	0.10
	In rigor	0.13	0.25	0.08	0.15
No. 54c	Normal	0.26	0.10	0.07	0.10
	In rigor	0.06	0.23	0.08	0.14
	Normal	0.22	0.13	0.08	0.11
	In rigor	0.05	0.25	0.06	0.13
Average	Normal	0.26	0.10	0.07	0.10
	In rigor	0.07	0.28	0.07	0.14
	Difference	−0.19	+0.18	±0	+0.04

method was used for determining lactic acid. The remainder of the hydrochloric acid extract was used for determining elemental phosphorus by the Bell-Doisy-Briggs (1922) method and for estimating the free sugar content; but in all probability the values obtained for free sugar are too high

because the extract was not further purified by the alumina cream and lead acetate method recommended by Macleod.

1. Normal bull frogs. Table 1 shows the results of analyses of the muscles of normal bull frogs. The glycogen content of the gastrocnemius averages 0.37 per cent, and of the thigh muscles 0.25 per cent. This is considerably less than that found in our previous work on Rana pipiens, in which species the glycogen content of the gastrocnemii averaged 3 per cent, and that of the thigh, 2 per cent. The lactic acid content of resting muscles was found to be in the neighbourhood of 0.1 per cent. The free sugar averaged 0.06 per cent; the phosphorus 0.1 per cent. It can be seen from the table that these figures are constant for different individuals.



After stimulation to fatigue, the gastrocnemius lost 0.21 per cent glycogen, and gained 0.21 per cent lactic acid. The other substances remained practically unchanged. The fatigue tracing of frog 52 is reproduced in figure 1.

After the muscles had passed into rigor, the glycogen loss was found to be 0.19 per cent, and the lactic acid gain 0.18 per cent. The free sugar was unaltered; the phosphorus showed a slight but definite (0.04 per cent) tendency to increase.

2. Validity of comparing different muscles. A comparison of corresponding muscles from opposite legs of the same frog, treated similarly, shows that differences can be regarded as significant only when they exceed the following values: glycogen, 0.1 per cent; lactic acid, 0.09 per cent; free sugar, 0.02 per cent; phosphorus, 0.03 per cent. These figures represent the var-

iation possible between corresponding portions of muscle on opposite legs of frogs which have just died, but it may be seen from table 2 that as a rule variation is much less than this. Provided the quantities of muscle analysed are over one gram in weight, it is justifiable to regard the chemical content of corresponding muscles in the same frog as being approximately the same. In frog 49 (table 2), the large difference observed in the glycogen and lactic acid content of the first thigh muscle was in all probability due to the use in one case of a portion of muscle weighing only 0.6 gram.

TABLE 2

Dead bull frogs

Concentration of substances in muscles on opposite legs of same bull frog

FROG		GLYCOGEN	LACTIC ACID	FREE SUGAR	PHOSPHORUS	REMARKS
No. 49	Gastrocnemii	0.12	0.10	0	0.17	Just dead. No rigor mortis
		0.09	0.13	0.06	0.17	
	Thigh muscles	0.06	0.07	0.04	0.14	
		0.16	0.16	0.05	0.16	
	Thigh muscles	0.11	0.07	0.03	0.14	
		0.11	0.08	0.06	0.17	
Average difference		0.02	0.04	0.04	0.02	
No. 51a	Gastrocnemii	0.09	0.10	0.18	0.04	Dead, in rigor mortis following insulin convulsions
		0.05	0.02	0.40	0.09	
	Thigh muscles	0.02	0.10	0.11	0.06	
		0.05	0.10	0.11	0.06	
	Thigh muscles	0.02	0.08	0.11	0.06	
		0.02	0.	0.22	0.05	
Average difference		0	0.5	0.11	0.02	

3. Bull frogs after convulsions (not treated with insulin). There were four bull frogs which died soon after arrival, there being no apparent reason for their death. Before death they passed through convulsions closely resembling those produced by the injection of insulin. Three of these frogs were taken while their hearts were still beating slowly and their muscular responses to electrical stimulation were still vigorous, and were treated as normal bull frogs and their muscles analysed. The findings are tabulated in table 3. The heart of the fourth frog had stopped beating, and the muscles gave no response to electrical stimulation, but there was no evidence of rigor. Its muscles were analysed without being fatigued or sent into rigor, and the results are tabulated in table 2.

It will be seen in table 3 that the glycogen content of the resting muscles is very low (0.05 per cent), while the lactic acid content is rather high

TABLE 3

Bull frogs after convulsions (not treated with insulin)

FROG		GLYCOGEN	LACTIC ACID	FREE SUGAR	PHOSPHORUS
Concentration of substances in gastrocnemii before and after electrical stimulation to fatigue					
No. 50a	At rest	0.05	0.23	0.09	0.14
	Fatigued	0.05	0.22	0.07	0.13
No. 50b	At rest	0	0.18	0.05	0.10
	Fatigued	0.02	0.26	0.04	0.12
No. 54a*					
Average	At rest	0.03	0.21	0.07	0.12
	Fatigued	0.04	0.24	0.06	0.13
	Difference	+0.01	+0.03	−0.01	+0.01
Concentration of substances in thigh muscles before and after chloroform rigor					
No. 50a	Flaccid	0.05	0.18	0.06	0.14
	In rigor	0.05	0.18	0.05	0.11
	Flaccid	0.02	0.21	0.06	0.15
	In rigor	0.06	0.23	0.07	0.15
No. 50b	Flaccid	0.02	0.15	0.05	0.10
	In rigor	0.01	0.31	0.06	0.17
	Flaccid	0.01	0.19	0.04	0.11
	In rigor	0	0.25	0.05	0.14
No. 54a	Flaccid	0.23	0.05	0.08	0.09
	In rigor	0.02	0.25	0.12	0.14
	Flaccid	0.17	0.12	0.06	0.10
	In rigor	0.09	0.30	0.09	0.11
Average	Flaccid	0.08	0.15	0.06	0.12
	In rigor	0.04	0.25	0.07	0.14
	Difference	−0.04	+0.10	+0.01	+0.02

* Gastrocnemius missing.

(0.18 per cent). The free sugar and phosphorus values are the same as as those found in normal bull frogs (table 1).

TABLE 4

Bull frogs after insulin convulsions

FROG		GLYCOGEN	LACTIC ACID	FREE SUGAR	PHOSPHORUS
Concentration of substances in gastrocnemii before and after electrical stimulation to fatigue					
No. 51b	At rest	0.03	0	0.29	0.05
	Fatigued	0.01	0.02	0.47	0.10
No. 53a	At rest	0	0.13	0.03	0.08
	Fatigued	0	0.18	0.07	0.14
No. 53b	At rest	0.13	Lost	0.06	0.18
	Fatigued	0.06	0.08	0.06	0.10
No. 55a	At rest	0	0.04	0.06	0.12
	Fatigued	0	0.06	0.05	0.12
No. 55b	At rest	0.12	0.09	0.07	0.10
	Fatigued	0.06	0.24	0.06	0.11
Average	At rest	0.05	0.05	0.10	0.11
	Fatigued	0.03	0.11	0.14	0.11
	Difference	−0.02	+0.06	+0.04	±0
Concentration of substances in thigh muscles before and after chloroform rigor					
No. 51b	Flaccid	0.02	0.10	0.02	0.14
	In rigor	0.02	0.03	0.16	0.06
	Flaccid	0.03	0.02	0.39	0.07
	In rigor	0.02	0	0.46	0.06
No. 53a	Flaccid	0	0.06	0.04	0.12
	In rigor	0	0.12	0.04	0.11
	Flaccid	0	0.03	0.02	0.06
	In rigor	0	0.06	0.04	0.15
No. 53b	Flaccid	0	0.14	0.07	0.11
	In rigor	0	0.25	0.06	0.19
	Flaccid	0	0.09	0.01	0.11
	In rigor	0	0.17	0.05	0.14
No. 55a	Flaccid	0	0.11	Lost	0.11
	In rigor	0	0.07	0.05	0.13
	Flaccid	0	0.02	0.04	0.10
	In rigor	0	0.04	0.04	0.12
No. 55b	Flaccid	0.03	0.06	0.06	0.09
	In rigor	0.01	0.18	0.05	0.12
	Flaccid	0	0.06	0.07	0.09
	In rigor	0.02	0.16	0.05	0.12
Average	Flaccid	0.01	0.07	0.07	0.10
	In rigor	0.01	0.11	0.10	0.12
	Difference	±0	+0.04	+0.05	+0.02

After fatigue or rigor the glycogen lost is practically nil (0.01 per cent), while the lactic acid gained is 0.07 per cent. The free sugar remains unchanged while the gain in phosphorus is trifling (0.02 per cent).

4. Bull frogs after insulin convulsions. Of six frogs injected with insulin, five were taken immediately after insulin convulsions and submitted to the usual treatment. The findings are recorded in table 4. The sixth frog was found dead, in marked rigor; its muscles were analysed for purposes of comparison and the determinations are given in table 2.

In "resting" muscles the glycogen content varied from zero to 0.13 per cent, with an average of 0.03 per cent. The lactic acid averaged 0.06 per cent and was even less than this in the muscles of the frog found in rigor mortis. The free sugar and phosphorus were almost the same as in normal resting muscles.

The loss of glycogen following electrical stimulation or rigor was practically nil, there being very little to lose in the first place. The gain in lactic acid is greater than might be expected, but very slight when compared with the gain in normal muscles. The changes in free sugar and phosphorus content are not really significant although there appears to be a tendency for both substances to increase during stimulation or rigor.

The fatigue tracing of frog 53b is shown in figure 2, and is representative of this class of frog.

CONCLUSIONS

1. Normal muscles, when subjected to electrical stimulation or to chloroform rigor, show a loss of glycogen which is exactly balanced by a gain in lactic acid.

2. Provided that quantities over one gram in weight are used for analysis, corresponding muscles on opposite legs of the same frog may be used for purposes of comparison in detecting changes in lactic acid and glycogen.

3. Frogs suffering from idiopathic or insulin convulsions are found to have a very low content of glycogen—sometimes indetectable by our methods. Consequently, when they are fatigued or made to pass into rigor, they exhibit very trifling losses of glycogen and gains in lactic acid, the increase in the latter substance, however, being greater than the loss of the former. Muscles in rigor mortis following insulin convulsions contain very little lactic acid (0.05 per cent).

4. Excess of lactic acid gained, over glycogen lost, cannot be accounted for by changes in the free carbohydrate or phosphorus content of the muscle.

5. Isolated muscles in which little or no glycogen can be detected by our method, can nevertheless be made to contract by electrical stimulation, frequently showing a fatigue curve similar to that traced by a normal muscle.

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ANALYSIS OF MUSCULAR RESPONSES OCCURRING DURING CONVULSIONS OF CENTRAL ORIGIN[1]

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The utilization of aperiodic isometric levers in the study of muscular activity has materially aided in the analysis of muscular contraction and reflex action. The method has been recently applied for this purpose by a number of workers, notably by Sherrington and by Fulton, in whose hands it has resulted in important additions to our knowledge. It is the purpose of this paper to present the results and to give an analysis of the data obtained through the application of the isometric lever to a study of muscular responses resulting from central stimulation by various drugs.

The observations were all made on decerebrate frogs, prepared by crushing the fore part of the brain with strong forceps, thus minimizing hemorrhage and avoiding injury to the medulla. In mounting the preparation it is essential that the entire system be rigid otherwise the record cannot give a true picture of the activity of the muscle. The frog was pinned to a special board which was securely attached to a heavy adjustable stand. The lever, a torsion wire myograph, made in accordance with the specifications given by Fulton (1925), was mounted on the same stand. Most of the myograms were recorded from the gastrocnemius muscles, and in these experiments the knee joint was firmly fixed by passing a pin through it and through two upright supports, which were part of an adjustable platform mounted on the frog board. By means of a thumb screw beneath the board this platform could be raised or lowered, thus varying the distance between the attachment of the muscle at the knee joint and the point of fixation of the tendonous end on the lever. This gave an arrangement by which any initial tension could readily be produced and maintained throughout the course of an experiment. In many preparations electrodes were placed on the sciatic nerve, either close to its emergence from the cord or more peripherally in the thigh, in order to make possible the comparison of the results of nerve stimulation with contractions of central origin. A second adjustable platform was mounted on the same board, and a second

[1] A preliminary statement giving a summary of part of the experiments recorded in this paper was published in the Journ. Pharm. Exper. Therap., 1927, xxxi, p. 227.

myograph on the stand to make possible simultaneous records of the two gastrocnemius muscles or of the flexors and extensors. Two methods of recording were used, *a*, by the attachment of a light straw to the isometric lever, ordinary kymograph records were traced on smoked paper, and *b*, by the use of a small mirror placed at the axis of rotation of the wire, a beam of light was reflected into a camera and recorded on a moving strip of bromide paper.

The following procedure was followed in a majority of the experiments: The frog is prepared and placed on the board as indicated. The tendon of the muscle to be studied is attached to the lever by a short piece of wire, at first without tension on the muscle. With the pointer resting on the smoked paper the drum is given one complete revolution, which traces a line representing zero tension. The muscle is then subjected to a small tension, usually about 50 grams, by dropping the platform on the board by means of the thumb screw, and the drum again revolved by hand, thus giving a second line representing the initial tension. This line is retraced during the course of the experiment, during which muscular activity is recorded. Frequently during intervals of relaxation, the platform is adjusted to compensate for any stretching of the muscle, i.e., the pointer is brought back and maintained at the line representing the initial tension. Various modifications of this procedure which were employed from time to time will be described in connection with the presentation of the data. Several convulsive drugs have been utilized in the course of this investigation, but the present paper will be confined for the most part to the results obtained by the use of picrotoxin, which was employed in doses of 0.006 mgm. dissolved in normal saline and injected in the ventral lymph sac.

General character of the tracings. The myograms recorded following the injection of the drug afford an excellent means of studying the development and general character of the convulsions. Picrotoxin belongs to the group of convulsant poisons which act primarily on the medulla. The convulsions continue after destruction of the fore brain but cease following removal of the medulla. Cushny (1918, p. 283) states that in the frog there is also some increase in the irritability of the spinal cord, a fact which is readily observed in the increased reflexes following the injection of picrotoxin in spinal preparations.

The first recordable contractions start in from eight to twelve minutes following the injection of picrotoxin in the doses used; an interval that is susceptible, however, to great prolongation in cases where there has been previous injury to the circulation. For example, in one series of ten experiments in which electrodes had been placed on the nerve roots in the abdominal cavity before the injection, the average time elapsing before the first convulsion was approximately seventeen minutes. The con-

vulsions are of short duration usually lasting from 5 to 10 seconds, followed by a period of quiescence, thus giving a rhythmic character to the records. In the beginning the tension developed is very small, but with each succeeding convulsion it becomes greater, until finally a maximum is reached in the course of 5 to 20 minutes, following which there is a gradual subsidence. Successive convulsions may occur in a very irregular fashion both as regards time and extent, but, on the other hand, a large number of preparations have given records of remarkable regularity, the intervals between convulsions being uniform and the tension developed climbing gradually

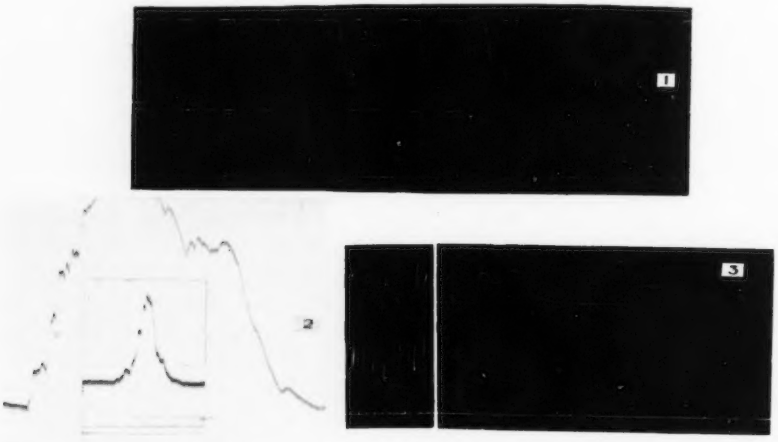

Fig. 1. Activity of the two gastrocnemii simultaneously recorded following the subcutaneous injection of picrotoxin. In this and the other figures the upper line in each tracing represents zero tension and the lower line the initial tension upon which are superimposed the records of muscular activity. Bottom record gives the time in 6-second intervals.

Fig. 2. Two examples of the action of the sartorius muscle photographed during picrotoxin convulsions. Time in 1-second intervals.

Fig. 3. Contractions of the isotonically arranged tibialis anticus (above) and the gastrocnemius (below). The second part of the tracing was recorded on a faster drum. Time recorded in 6-second intervals.

to a maximum and then falling off. Frequently an irregularly sustained contraction lasting from 10 to 20 seconds occurs at the time of the greatest tension development, and at this time there is a tendency for the convulsions to be more frequent and more prolonged.

Typical records showing the development and nature of the contractions of the gastrocnemius muscle during picrotoxin convulsions are reproduced as figures 1 and 5. Since these records were obtained through an isometric lever the tension at any point on the tracing can be expressed in grams

and, on the basis of the all or none law, the height of the curve above the line of zero tension is proportional to the number of active fibers. There are two factors which conceivably may modify the efficiency of individual fibers and thus effect this relationship to a slight extent; first, the possibility suggested by Katz (1925) that the gastrocnemius not being a parallel fibered muscle the direction of pull of any fiber may vary depending on the number of active fibers in the whole muscle, and, secondly, the fact pointed out by Fulton (1926, p. 187) that the greater the tension developed by the muscle the greater will be the shortening of the active fibers, and therefore each fiber will give less tension. These factors however can be ignored in the present study because even should they appreciably affect the recorded tensions the general relationship would still hold, i.e., the greater the tension recorded the greater the number of active fibers.

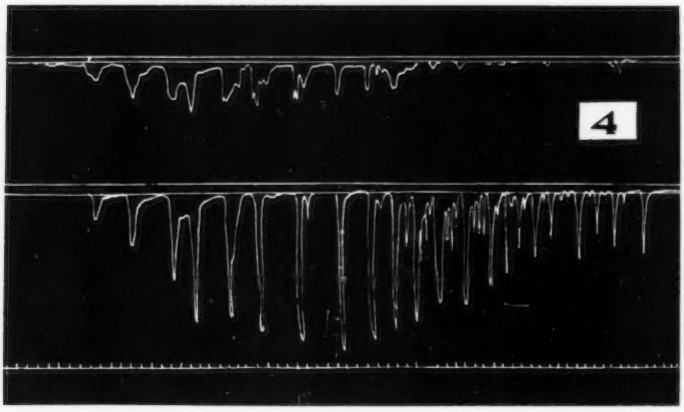

Fig. 4. Same as figure 3, but with an isometrically arranged muscle. Time in 3-second intervals.

It is apparent from the records that in the early stages of the convulsions relatively few fibers are simultaneously active, but subsequently more and more take part until the maximum effect is reached. The decrease in maximum tension which follows in later convulsions does not necessarily mean that the discharge from the centers involves fewer fibers for here, as we shall see later, we have the factor of peripheral fatigue beginning to influence the magnitude of the tensions developed.

In many records the early contractions following the administration of picrotoxin show an irregular step like character in both the rising and falling phases of the curve, presumably due to the accession and dropping out of new groups of active fibers. In order to study the phenomenon in

more detail, arrangements were made for recording the isometric contractions of the sartorius muscle through the optical system already described. The sartorius was chosen because it is a small muscle containing relatively few fibers and the optical method of recording permitted of large magnification of tension changes without distortion. Examples of records so obtained are given in figure 2. With the magnification used the maximum tension recorded in the smaller of the two tracings represents only about one-tenth the total of which the muscle was capable. The characteristic checks during the development and loss of tension are apparent, giving clear evidence that at the onset of convulsions, all the fibers of a muscle are not stimulated simultaneously, but rather there is a gradual recruit-

Fig. 5. Tracings obtained simultaneously from the left and right gastrocnemii showing similarity in action of the two muscles throughout the course of convulsions.

Fig. 6. The activity of the two gastrocnemii during picrotoxin convulsions compared with the results of nerve stimulation. The arrows point to the responses resulting from the application of short periods of tetanic stimuli to the nerve.

ment of additional fibers, and also a gradual falling out of active fibers during the subsidence of the convulsion. These checks in the curves are in many cases numerous enough to suggest the possibility that each one represents the addition or cessation of activity of a group of muscle fibers innervated by a single nerve fiber. The fact that the step like character of the curve is more or less obliterated in the later stages of the experiment is doubtless to be explained by the onset of fatigue, and consequent failure of the muscle fiber to maintain tension, i.e., there is a gradation in the work of the individual fiber.

The action of antagonistic muscle. A number of experiments were carried

out in which the activity of the gastrocnemius and the tibialis anticus muscles of one leg were simultaneously recorded in a frog undergoing picrotoxin convulsions. Typical records are reproduced in figure 3 and figure 4 obtained respectively with an isotonic and an isometric lever. No evidence of reciprocal action has been observed, but, on the contrary, during a convulsion both muscles are commonly active at the same time, and they both follow the general rhythm of successive convulsions. However, the tension at any one time does not correspond in the two muscles, so there are changes in the number of active fibers in the antagonistic muscles with relation to each other, which probably account for the irregular movements of the limbs taking place during convulsions in the intact frog.

Symmetry of central discharge. When the contractions of the two gastrocnemii are simultaneously recorded it is found that in general they are active at the same time. See figures 1 and 6. This is merely a reflection of the usual course of picrotoxin convulsions; periods of generalized muscular activity being alternated with periods of quiescence. In many cases, however, the correspondence in the activity of the two sides is much closer as, for example, in the experiment from which figure 5 is reproduced. Here the similarity in the tracings is very striking. Since the tension at any instant is determined by the number of fibers active, this result indicates a highly organized neuro-muscular system and a remarkable symmetry of central discharge. This similarity of the tension curves developed by the two muscles extending even to their finer details, along with the fact that the gastrocnemius muscle presents a complex arrangement of its fibers, strongly suggests that corresponding fibers in the two muscles are coördinated in their activity.

In the normal activities of the frog the corresponding muscles of the two hind legs are in general required to act in unison, whereas in many other animals they contract alternately. This is also true of certain other muscles of the frog. It would be interesting to know whether a similar symmetry of action could be demonstrated for these muscles, or whether perhaps the converse would be true. It is planned to study this point further.

Number of fibers simultaneously active during convulsions. By placing electrodes on the intact sciatic nerve in the thigh and applying a short tetanic stimulus of such strength that maximum response is obtained in the gastrocnemius muscle, it was possible to compare the maximum tension with that developed during central stimulation through the action of picrotoxin and other drugs. The results from eight experiments are summarized in table 1. The first column under the heading of maximum tension gives the tension developed by nerve stimulation in the fresh preparation, while the second column gives the maximum spontaneous

contraction occurring at any time in the course of the experiment, usually in from 15 to 25 minutes after the injection of the picrotoxin. In a majority of the experiments the maximum tension during convulsions approaches but does not equal that from nerve stimulation; the latter showing an average superiority of about 6 per cent. In experiments 7 and 8, however, actually more tension developed at the height of the convulsions than could be obtained from maximum tetanic stimulation of the sciatic nerve at any time during the course of the experiment. This comparison is not quite fair for it is probable that an even more marked discrepancy would be shown if it were not for the fact that the tension from artificial stimulation is recorded in fresh preparations, whereas a considerable period elapses before the activity of the muscle due to picrotoxin reaches its maxi-

TABLE 1

Comparisons of the tensions developed as a result of picrotoxin convulsions and nerve stimulation

EXPERIMENT	MAXIMUM TENSION		AFTER PARTIAL FATIGUE		
	Stimulation	Convulsion	Stimulation before convulsion	Convulsion	Stimulation after convulsion
	grams	*grams*	*grams*	*grams*	*grams*
1	495	435	495	435	335
2	565	415	318	338	300
3	463	360	383	320	308
4*	283	255	245	240	230
5*	300	240	243	235	228
6	525	505	475	505	378
7	500	538	500	638	485
8	650	725	650	725	535
Average....	475	447	414	429	350

* Data recorded for function of VIIIth root only.

mum, and by this time the intervention of fatigue has reduced its power. Similar figures from other experiments relating to the development of fatigue are included in table 2.

In the production of convulsions by picrotoxin, accurate control of their time of onset and of their severity is not possible, and therefore the tension developed by the muscle at any given moment may not represent a condition of maximum activity. Weak convulsions can not be evaluated with reference to any standard, but when the tension approaches that obtained from nerve stimulation significant conclusions may be drawn. For this reason it is justifiable to select from the records of each experiment the tracing indicating the highest tension in relation to the results from nerve stimulation. This has been done and the results recorded in the last three columns of table 1. The middle column of the three represents

the tension developed during a convulsion of marked intensity, the first column the tension obtained on nerve stimulation applied a short time previously (stimuli were applied at regular intervals throughout the course of each experiment), and the last column the tension resulting from stimuli applied immediately on the cessation of the convulsion. These data are necessarily taken from the tracings at a point representing a period of from 15 to 25 minutes after the injection of picrotoxin when the preparation is in a partially fatigued condition.

The table shows a number of instances where the tension developed during convulsions in the partially fatigued muscle is greater than that obtained just previously from nerve stimulation, and the average for the eight experiments gives a small excess in favor of central stimulation. When account is taken of the rather rapid development of fatigue which must have progressed to some extent between the moment of the last previous stimulation and the onset of the next strong spontaneous activity this difference assumes greater significance. In the last column of figures are given the results of stimuli applied shortly after the convulsion, and here the discrepancy of about 19 per cent must be due in part to fatigue. The phenomenon of the tension from central stimulation exceeding that which it has been possible to obtain by stimulation of the nerve has also been obtained in convulsions caused by the injection of veratrine.

These results present two points of interest. The fact that the tensions developed at the height of the convulsions approximate those obtained by the application of a maximum stimulus to the nerve indicates that during such a convulsion every muscle fiber is simultaneously active; a condition which does not ordinarily occur during reflex activity (see Camis 1909; Fulton 1926, p. 154; Cooper, Denny-Brown and Sherrington, 1926; and Forbes, Whitaker and Fulton, 1927). Secondly, how are we to account for the occasions when the tension developed by the muscle during a convulsion exceeds that which it is possible to obtain by nerve stimulation? This unexpected result suggests that some nerve fibers have escaped stimulation, but careful search has failed to confirm this suspicion. The only alternative explanation, in keeping with the all or none law, is that the tension is in some way modified by the interval between nerve impulses passing down the individual axons. It is tentatively suggested that the rate of central discharge may be such that each succeeding impulse falls in the super-normal phase (Lucas, 1917, p. 45) and thus their passage across the partially fatigued neuro-muscular junction is facilitated, with the result that more muscle fibers are stimulated than is the case with the less favorable rate employed in artificial stimulation. The problem requires further study with accurately controlled rates of tetanic stimuli.

The location and extent of fatigue resulting from central convulsions. As has already been indicated the tension developed during convulsions, after

reaching a maximum, gradually declines and this is true, but to a lesser degree, when further successive injections of picrotoxin are given. Some insight into the place of onset of this failure, or fatigue, was obtained by the application of tetanic stimuli to the nerve at various times during the course of the experiments, and comparing the resulting tensions with those of central origin. A record showing part of a tracing from such an experiment is shown in figure 6, and the data from four experiments are given in table 2.

In this series of experiments electrodes were applied to both the VIIIth and IXth spinal nerve roots in the abdominal cavity. One or the other of these two roots was cut close to its emergence from the vertebral column, thus protecting the muscle from stimulation through this approach. The

TABLE 2

Comparison of the maximum tensions in grams developed as a result of picrotoxin convulsions and nerve stimulation during the course of the development of fatigue

EXPERIMENT	BEFORE DRUG		MAXIMUM	15 MINUTES AFTER DRUG			30 MINUTES AFTER DRUG	
	Stimulation		Convulsion	Stimulation		Convulsion	Stimulation	
	Cut root	Intact root	Intact root	Cut root	Intact root	Intact root	Cut root	Intact root
1	624	693	486	603	480	486	534	327
2	474	687	684	456	654	651	300	390
3	468	807	867	366	633	546	318	297
4	696	360	294	666	237	234	654	234
5	255	648	612	234	450	426	222	366
6	120	594	414	111	294	267	102	264
Average	440	632	560	406	458	435	355	313

tension developed by the muscle on artificial stimulation of this root made a satisfactory standard for a comparison of the fatigue resulting from activity induced by central stimulation, and served as a control with reference to possible deterioration of muscle tension from a direct influence of the drug or other causes. This control is not invalidated by the probable presence of pleurisegmentally innervated fibers, for it has been shown (Samojloff, 1924; Beritoff, 1924; Cattell and Stiles, 1924), that fatigue resulting from stimulation of one root results in but slight modification of the tension developed on stimulation of the other root.

Reference to the figures given in table 2 shows that in general as the convulsions subside there is a corresponding decrease in the tension developed on electrical stimulation of the nerve. This loss of power at the time when spontaneous activity had practically ceased, amounted on the average to more than 50 per cent of the original performance of the muscle, and could be further reduced by additional picrotoxin injections. The

function of the fibers innervated by the control root shows an average loss in the course of the experiments of only thirteen per cent. A second series of experiments was carried out in which simultaneous records were obtained from the two gastrocnemii, during the course of which stimuli were applied at regular intervals to the whole sciatic nerve on both sides, one, with cut sciatic, serving as a control. In five experiments the average power of the muscle was reduced to less than a third (31.6 per cent) during the course of the convulsions, while the control muscles, protected by nerve section, retained in the average 96 per cent of their original power at the end of the experiment. Other preparations in which veratrine was used as the convulsive agent gave closely comparable results.

In the case of strychnine the degree of tension developed during reflex tetanic convulsions was never exceeded by nerve stimulation at any time in the course of an experiment. Another point of interest is that, in a frog under the influence of this drug in suitable concentration, at the time reflex activity ceases no further muscular contractions can be brought forth by direct stimulation of the nerve. These results will form the subject of another paper. They serve to direct attention to the periphery as the probable source of paralysis occurring in the later stages of strychnine poisoning, and throw doubt on the usual statement that the centers are at fault.

The general trend of these results indicates that during convulsions of central origin a considerable proportion, possibly all, the efferent nerve fibers to the gastrocnemius muscle of the frog, including their connections in the anterior horn cells continue to function longer than the peripheral mechanism. It thus appears that with regard to the primary site of fatigue the influence of central stimulation by chemical substances differs from that of reflex activity in a striking manner. In the case of fatigue induced by the repetition of some reflex it is the centers which are particularly susceptible and first fail to transmit the impulse. Moreover, the work of Sherrington (1906, p. 218) and of Forbes (1912) has shown that after a reflex can no longer be obtained from a stimulus at a given location because of frequent repetition, it may again be elicited when the stimulus is shifted so that the impulse travels along a new afferent approach, and since the same motor cells are involved in each instance the results point to a primary fatigue in the synaptic connections to these cells. In the case of picrotoxin convulsions, however, where the impulse presumably has its origin in the motor side of the arc in the medulla, the same synapses connecting the anterior horn cells would be involved throughout the duration of the convulsions, but, in spite of this, they continue to function for a long period—so long, that the action of the peripheral mechanism is greatly reduced. At first sight this appears to oppose the evidence of the authors just cited, but Doctor Forbes has pointed out to me that this is not necessarily the case, for there may be

complications arising from an influence of the drug employed on the structures of the cord. There is evidence that the three substances used in the present experiments, picrotoxin, veratrine and strychnine, all increase the excitability of the spinal cord. This being the case it is not improbable that they serve to delay the onset of fatigue in certain synapses normally resulting from the extensive conduction of impulses arising in higher centers. On this basis it may be assumed the normal sensitivity of the spinal cord mechanism to fatigue protects the peripheral neuro-muscular apparatus from exhaustion, whereas the stimulating action of picrotoxin and other drugs on the cord allows of a continuation of the passage of impulses along the central pathways so that the activity of the peripheral structures may be continued until almost complete loss of function results.

Location of fatigue in the peripheral mechanism. Having established that the decline in the power of a muscle which takes place during long con-

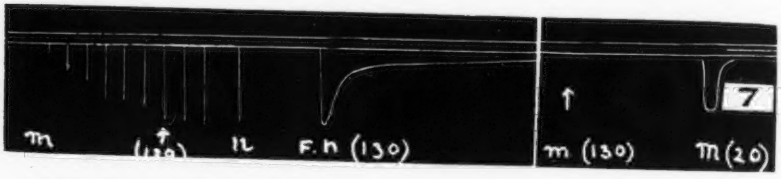

Fig. 7. Illustrating the procedure and results of an experiment relating to the influence of prolonged nerve stimulation on the response of the muscle to direct stimulation. Numerals indicate coil positions. *m*, responses to a series of stimuli applied directly to muscle to determine position of coil giving a maximum response—in this case 130 mm. *n*, same strength of stimulus applied to nerve. *F. n. (130)* fatigue curve caused by nerve stimulation. *M (130)* response of muscle to direct stimulation with same strength of current after fatigue, and *m (20)* response of muscle to strong direct stimulation.

tinued picrotoxin convulsions is primarily due to changes in the peripheral mechanism, it is of interest to inquire whether this loss of power is the result of a failure of the impulse to get past the end plate or of deterioration in the contracting mechanism of the muscle fiber. Some idea of the relative importance of these factors has been obtained by stimulating the nerve until no further tension is developed by the muscle, and then quickly shifting the stimulus so that it acts directly on the muscle. It is well known that with isotonic levers very little change in the extent of shortening following direct stimulation of the muscle occurs as a result of prolonged nerve stimulation. A number of tests have been made with the isometric lever in accordance with the following procedure: The position of the coil giving a just maximum response was first determined, then, without changing the strength of the stimulus, the nerve was stimulated tetanically until the muscle completely lost its power of producing tension

The stimulus was now shifted back so as to act directly on the muscle. In eight such experiments practically no improvement occurred on shifting the stimulus, i.e., the muscle stimulated to complete "fatigue" through its nerve was not able to respond to the direct action of a stimulus of an intensity formerly producing a maximum response. We are not warranted, however, in drawing the conclusion that the individual muscle fibers are completely exhausted following the long continued nerve stimulation for the loss of power exhibited might well result from an increase in the threshold of the muscle so that the given stimulus becomes inadequate. Actually this is found to be an important factor, for when a very strong stimulus is applied to the muscle it will again respond with a considerable development of tension. A typical tracing is reproduced in figure 7. In the case of a just maximum stimulus there can be no question but what its distribution throughout the muscle mass is aided by the nerve fibers within the muscle, and the loss of this influence following nerve stimulation results in an apparent rise in the threshold of the muscle. In the eight above mentioned experiments it was possible, by pushing the secondary coil of the inductorium to a position giving very strong stimulation, to bring forth an average tension equalling 73.7 per cent of that obtained in the fresh muscle.

This result indicates that changes of threshold in the muscle or in the neuro-muscular junction, rather than a failure of the contracting mechanism, account for the greater part of the decline occurring as a consequence of prolonged nerve stimulation. Taken with the evidence presented in earlier parts of this paper they lead to the conclusion that it is the region of the neuro-muscular junction which first ceases to function in prolonged convulsions produced by the central action of picrotoxin and certain other drugs.

SUMMARY

1. Tension changes developed by the gastrocnemius and other muscles in decerebrate frogs following subcutaneous injection of picrotoxin were recorded by means of a torsion wire isometric lever. In a short period after the drug is injected rhythmic contractions of the muscle occur which increase in strength and frequency until a maximum is reached when they slowly decline, all recordable activity ceasing in from one-half to one hour.

2. Simultaneous records taken from the two gastrocnemii frequently result in closely similar tracings, indicating a correspondence in the number of muscle fibers contracting on the two sides and a striking symmetry of central discharge.

3. Activity of the antagonistic flexor muscle is associated with a contraction of the gastrocnemius. The relative tensions between the two groups is variable, but no reciprocal relation has been demonstrated.

4. The tension developed at the height of a convulsion approximates that obtained by the application of a maximum tetanic stimulus to the nerve, indicating that during such a convulsion nearly all the fibers of a muscle are active.

5. Occasionally in the partially fatigued gastrocnemius muscle the tension developed during a severe convulsion exceeded that which it was possible to obtain from direct tetanic stimulation of the sciatic nerve.

6. As the picrotoxin convulsions subside and the tensions recorded become smaller there is a corresponding decrease in the tension developed on electrical stimulation of the nerve. This represents a loss of approximately two-thirds of the original pull of the muscle at the time when spontaneous contractions cease, and indicates that under these conditions a considerable proportion, if not all the motor nerve fibers, including their connections in the anterior horn cells, continue to function longer than the peripheral mechanism. A similar general result was obtained in convulsions due to veratrine and strychnine.

7. The isolated muscle stimulated to complete "fatigue" through its nerve will still exhibit on the average nearly three-fourths of its former power when stimulated directly with a strong current, indicating that the loss of tension is due in large measure to an increase in the threshold of excitability, rather than to a failure of the contracting mechanism.

8. These observations lead to the conclusion that the region of the end plate is the first to cease functioning in prolonged convulsions resulting from the central action of picrotoxin.

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THE INFLUENCE OF THE VAGI ON THE MOTILITY OF THE EMPTY STOMACH IN NECTURUS

Comparative Studies VI[1]

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In a previous article (Patterson, 1916) I showed that the gastric hunger contractions in the bullfrog exhibit no periodicity, the contractions being practically continuous; that the simpler gastric mechanism of this animal does not show the distinction between the digestive peristalsis and the hunger contractions found in the higher animals; that the hunger contractions are inhibited or weakened by the introduction directly into the stomach of small quantities of water, weak alkali and acid; that when these substances are introduced directly into the filled stomach they inhibit the digestive peristalsis in the same order of magnitude as they inhibit the hunger contractions of the empty stomach; that during prolonged fasting the hunger activity is greatly augmented, the contractions showing a marked increase in their amplitude directly proportional to the length of the fast, but there is no increase in the gastric tonus; that within certain not very wide ranges of temperature the hunger movements fall within the limits of van't Hoff's rule; and that the stomach of the decerebrate animal behaves in a similar manner to that of the normal. From the standpoint of comparative physiology attention should be called to the fact that the stomach of higher animals exhibits a definite periodicity; that fluctuations in tonus occur with a marked increase during fasting; and that a marked distinction exists between digestion and hunger peristalses.

On completion of this work it seemed to me desirable to extend the investigation to necturus, a member of that group of aquatic amphibians which is probably the oldest of the present day urodeles (Swingle, 1922)

[1] Preliminary reports of this work were given before the American Physiological Society at Chicago, December 30, 1920, and at St. Louis, December 28, 1923, brief Abstracts of which were published in the Proceedings of that society. (This Journal, 1921, lv, 283 and Ibid., 1924, lxviii, 127.) Similar reports were also made before the Iowa Academy of Science at Mount Vernon, Iowa, April 27, 1923 (Proc. Ia. Acad. Sci. 1923, xxx, 185) and the Michigan Academy of Science, Arts and Letters at Ann Arbor, Michigan, April 3, 1924. (Proc. Mich. Acad. Sci. Arts and Letters, 1924, iv, 511.)

to determine whether or not the physiological activity and control of the gastric motor mechanism discovered in bullfrogs is the same or similar in this animal.

METHODS. *Necturus maculatus* was employed throughout this investigation. Over 160 animals were used. The general method of study at first was similar to that employed on the bullfrog (Patterson, 1916, 1920), modified to meet the anatomical peculiarities of this type of animal. The animals were anesthetized in a solution of 2 per cent ether in water after the method of Sulima (1909) and a stomostomy was made. This method was found to be unsatisfactory and although the animals lived indefinitely following the operation they invariably died after the introduction of the balloon tube through the stomostomy opening in the floor of the mouth, as for example, if the balloon and tube were left in position over night they were usually found dead in the morning. The presence of the tube in the opening apparently interfered with the normal respiration, thus allowing the passage of water into the mouth which could not be properly expelled through the gill slits, or the animals because of this abnormal condition may have drowned since water was found in most cases either in the lung sacs or in the stomach and upper portion of the intestine. No results were obtained by this method and it was discarded. A gastric fistula made in the mid-line of the ventral surface of the body also showed no improvement. The tissues were not sufficiently strong to hold the sutures for any length of time and they were soon torn out by the swimming activity of the animal and more especially if the abdominal muscles were quickly contracted resulting in a protrusion of more or less of the visceral mass. An esophageal fistula made about 0.5 cm. to the left of the mid-line and posterior to the fore limb on the ventro-lateral surface proved to be more satisfactory. By this method fairly good results were obtained from about one-third of the experimental animals but unfortunately the greater majority died of rupture as in the case of the gastrostomized animals before results could be obtained from them. However, such a fistula made in the extreme lower end of the esophagus was much more satisfactory than a similar one made in the mid-line, since there was less tendency to rupture due to the retaining of the visceral membrane which is attached to the inner surface of the abdominal wall along the mid-line. This sustaining membrane holds back the viscera of the right side and especially the large liver thereby reducing the intra-abdominal pressure on the tissues directly involved in the making of the fistula, thus lessening somewhat the possibility of rupture. Simply an attempt to introduce the balloon into the stomach of a properly prepared animal often results in rupture if the animal becomes excited and swims about in the water. The animals during the period of experimentation were kept in water in a darkened tank and in a reasonably cool room.

Because of the disadvantages involved in this method and other factors of an apparent inhibitory nature which will be discussed later, it was thought desirable to initiate a series of acute experiments on this animal.

In these experiments the method consisted of transecting the spinal cord between the first and second cervical vertebra under ether anesthesia followed immediately by a stomostomy operation for the introduction of the rubber balloon into the stomach via mouth and esophagus, for the graphic registration of the gastric contractions. The animal was then placed on an inclined board so that the gills floated in the 2 per cent ether and water solution and the vagi were isolated dorsally at their exit from the skull for a distance of 3 to 5 mm. through incisions about one centimeter to the right and left of the median line and lifting ligatures were placed under each nerve. These nerves can be isolated without hemorrhage. The incisions were plugged with cotton moistened with normal saline and the animals were then placed in a normal horizontal position on a specially constructed animal holder provided with water-troughs and the gills were constantly covered with running water, both from above and below. In these animals the respiration and circulation are fairly normally maintained for periods ranging from three to five days and they may live for periods as long as eight days. During this time the gastric motility as well as the influence of the vagi on the empty stomach may be studied.

Results. The recorded observations of the activity of the empty stomach of necturus by the esophageal-fistula method are similar to those recorded from the empty stomach of the bullfrog with some exceptions. The contractions, although somewhat weaker and of shorter duration, resemble in character those of the bullfrog but the intervals between the individual contractions are from five to nine times longer. Likewise, no changes in gastric tonus occur in these fasting animals and for this reason only one type of hunger contraction is exhibited since the type of these contractions is thought to be primarily dependent upon the degree of tonus under which the gastric mechanism maintains itself at different times (Rogers and Hardt, 1915). Under an environmental temperature of about 18°C. which has proven to be the most favorable for maximal gastric activity in this animal, the contractions show an average duration of about forty-five seconds and the intervals between the contractions vary from one and one-third to five minutes. The form of the contraction is rather slow and the curve is perfectly smooth showing no smaller superimposed waves. Furthermore, there is no indication that these contractions fall into groups separated by intervals of relative quiescence but they are practically continuous and show a definite regularity which continues for hours, at least after the contractions have once started following the preliminary steps of the introduction of the balloon into the stomach. Such procedure may result in total absence of any gastric activity for

several hours and when the contractions actually do start they are very, very feeble at first but show a definite regularity. They gradually increase in strength until they reach their maximum which may require from one to three or four hours. Weak acid or alkali of the usual strength when introduced directly into the stomach produces temporary gastric inhibition (Patterson, 1921a), as well as do body movements. From one animal a continuous record of the gastric activity of the empty stomach was recorded for seventeen and three-quarters hours in which no periodicity was exhibited, the contractions being regular and continuous. They would doubtless have gone on indefinitely under the conditions had not the temperature toward the end of this period been very gradually reduced by the addition of small pieces of ice to the water in the tank. This reduction in temperature produced a gradual slowing and weakening of the contractions until finally there was a complete cessation of all gastric activity which occurred at 7°C. This is a somewhat lower temperature than that required in the bullfrog to produce gastric standstill which was found to be upon an average 13°C. (Patterson, 1917). This was the only animal on which the influence of temperature was studied. The animal upon recovery showed feeble contractions which increased in intensity but they never quite returned to the normal strength of the contractions before the reduction of temperature. It should be borne in mind, however, that this particular experiment was carried out in the month of January while the similar and more extensive experiments on bullfrogs were carried out during the month of August and that might account in whole or in part, for the difference in temperature at which the stomachs of the two animals ceased their activity.

During the graphic registration of the gastric activity in necturus it was found that handling or touching the head and more especially the gills usually resulted in producing temporary gastric inhibition and this, coupled with the fact that it requires such a long time for the contractions to start after the introduction of the balloon into the stomach, might indicate that the gastric mechanism in this animal is under the inhibitory control of a medullary center. To determine this factor a series of acute experiments was started.

After the operative procedure necessary in preparing the animals for these acute experiments and the introduction of the balloon into the stomach there is usually a period of several hours before there is any indication of gastric motility. Finally, weak contractions appear which gradually increase in amplitude to a maximum. These contractions, on the whole, are practically identical with those obtained by the esophageal-fistula method. Previous reports (Patterson, 1923, 1924a, 1924b) indicate that the controlling influence of the vagi on the movements of the empty stomach of necturus is largely inhibitory. In this animal Fischer(1864),

Drüner (1901) and Norris and Buckley (1911) have described a glossopharyngeal-vagus nerve complex formed by three branchial nerves. The glossopharyngeal or first branchial nerve shows characteristic pharyngeal, pretrematic and posttrematic rami and sends a general cutaneous and motor branch that anastomoses with the second branchial nerve. The second branchial nerve (vagus 1) has well-developed pharyngeal, pretrematic and posttrematic rami and sends one motor and general cutaneous branch to supply the levator and depressor muscles of the first gill and the overlying skin which receives an anastomosis from the ninth nerve; other branches supply the levator and depressor muscles of the second gill; an anastomosis occurs with the third branchial nerve forming the innervation of the levator and depressor muscles of the third gill. The third

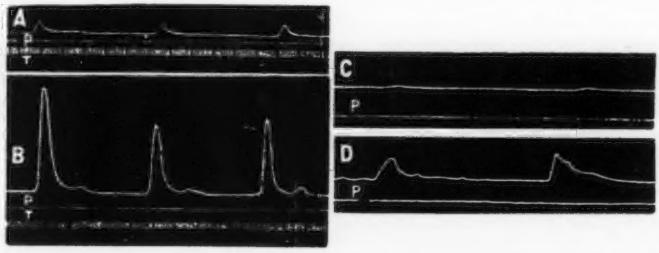

Fig. 1. Movements of the empty stomach of *Necturus* before and after double vagotomy. A, normal contractions with the vagi nerves intact; B, contractions from the same animal's stomach after section of both vagi. Note the augmentation of the contractions due to the removal of the inhibitory influence. C indicates how shallow the normal contractions may be in some animals followed in D by the usual augmentation after double vagotomy. P = 0 mm. pressure of water manometer. T, time in 5 second intervals.

branchial nerve (vagus 2) is very much reduced but pretrematic and posttrematic rami of communis fibers only may be recognized, while the main part of the nerve forms an anastomosis with a branch of the second branchial nerve already mentioned. The ramus intestino-accessorius of the vagus divides into three typical branches: r. lateralis ventralis, r. intestinalis recurrens, and r. intestinalis, the latter of which supplies the lungs, stomach and intestine. While the apparent close linking together of the respiratory and gastric organs might almost lead one to believe that in necturus they were both under the control of a single medullary center, it is more likely that separate centers exist and that reflex stimulation results. However, it is evident that this close relationship between the nerve supply of the gills and the stomach might be materially influenced by conditions affecting respiratory function which might be transmitted

to the gastric mechanism reflexly through this glossopharyngeal-vagus complex and the experimental results of this investigation tend to support this belief. Stimulation of the gills with a glass seeker or with forceps usually leads to reflex inhibition of the empty stomach with cessation of gill movement during the period of the excitation. The afferent impulses arising from gill stimulation pass along the branchial nerves to the vagus center in the medulla, whence inhibitory influence is transmitted down the vagus trunk and along the r. intestinalis to the stomach. Section of the branchial nerves abolishes the reflex effects of gill stimulation. Destruction of the medulla or section of the vagi produces a similar effect.

The ligaturing and sectioning of both vagi first produce inhibition of the gastric movements due to mechanical stimulation followed in a short time by the return of the gastric contractions in augmented form, the augmenta-

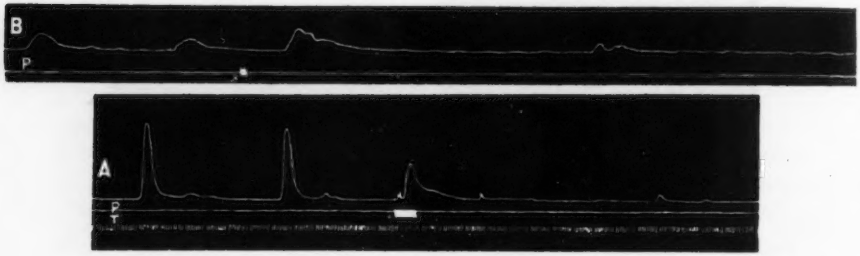

Fig. 2. Inhibition of the movements of the empty stomach of *Necturus*. A, same animal as in figure 1 A and B. At *X*, weak tetanizing current applied to peripheral end of left vagus after double vagotomy producing inhibition directly. B, same animal as in figure 1 C and D. At *X*, moderate traction on central end of right vagus after unilateral vagotomy producing inhibition reflexly. *P* = 0 mm. pressure of water manometer. *T*, time in 5 second intervals.

tion even greatly exceeding the amplitude of the normal contractions with the nerves intact (fig. 1 A, B, C, D). Electrical stimulation of the vagus or even slight traction on either the sectioned or the unsectioned nerve, when the stomach is exhibiting motor activity, will produce almost immediate inhibition or stoppage of the movements of the empty stomach (fig. 2 A). This inhibition is of rather prolonged duration lasting from 30 to 45 minutes or more, depending upon the intensity of the stimulation and is followed by a gradual return to normal. The effect is the same whether rapidly or slowly induced shocks are used but the duration of the inhibition is usually longer following stimulation by rapidly induced electric shocks. Cardiac inhibition also results from electrical stimulation of this nerve. No motor effects have ever been observed from vagus stimulation. These findings tend to show that the fibers contained in this

nerve and destined for the stomach of necturus are predominantly, if not exclusively, inhibitory. This predominant inhibitory action of the vagus on the stomach of necturus is not in accord with the results of other workers on the stomachs of higher animals, since it is now generally conceded that the vagi contain both motor and inhibitory fibers to the stomach with a predominance of the former. However, the work of Hopf (1911) on the frog and that of Bercovitz and Rogers (1921) on the turtle while in confirmation of the above statement seems to indicate that there is some slight increase in the proportionate number of inhibitory fibers present in the vagi to the stomachs of these animals as compared to the number existing in the same nerve of that of higher animals. Furthermore, Carlson and Luckhardt (1921) have demonstrated that the vagus nerve is inhibitory to the esophagus of the turtle. To the lungs which must be considered as diverticula of the esophagus it is predominantly, if not exclusively motor in the turtle (Carlson and Luckhardt, 1920a). Likewise, in the frog it is predominantly inhibitory but also partly motor to the lungs (Carlson and Luckhardt, 1920b; Patterson, 1921b) while in salamanders including necturus it appears to be exclusively inhibitory (Luckhardt and Carlson, 1921).

Stimulation of the central end of the vagus after unilateral vagotomy either with induced electric shocks or by traction leads to a reflex inhibition of the movements of the empty stomach (fig. 2 B). However, following bilateral vagotomy the reflex is entirely abolished showing the reflex pathway to be via vagus. This reflex activity on the gastric motor mechanism of necturus, as exhibited by central stimulation of the vagus and in most cases by stimulation of the gills in the intact animal is in accord with the results of other investigators on the reflex inhibition of certain internal organs in other animals. McWilliam (1885) working on the reflex excitation of the cardiac nerves of the eel, showed that various types of slight stimulation of either gill caused a sudden and powerful inhibition of the heart of considerable duration (half a minute) after the discontinuance of the gill stimulus. Stimulation of the gill apertures, the interior surface of the branchial chamber, the skin of the head and that of the tail are also effective in producing cardiac inhibition. Cardiac inhibition may also be obtained in the carp, perch, rudd, etc., as in the eel. Goltz (1872) also found that various types of stimulation applied to different parts of the frog's skin led to an increased hypertonus and motility of the stomach. These effects, however, he did not consider as types of reflex activity but presumed that the intense stimuli employed paralyzed more or less completely the medullary center from which under normal conditions the tonic inhibitory impulses for the esophagus and stomach arose. These observations were confirmed by Contejean (1892) and Steinach (1898). Meltzer and Auer (1906) showed that the vagus contains inhibitory fibers

to the cardia of the rabbit which may be excited reflexly by stimulation of the central end of the remaining vagus. Openchowski (1889a, 1889b) reports that, with the vagi intact, reflex dilatation of the cardia may be induced by stimulation of the sciatic nerve and various internal organs, while Luckhardt and Carlson (1920) by gentle mechanical stimulation applied to the skin of the mandible, gills or front legs of the axolotl induced lung contractions of reflex origin but such reflex action from electrical or mechanical stimulation of cutaneous nerves was not obtainable in the necturus. In the curarized cryptobranchus stimulation of cutaneous and visceral sensory nerves causes in most cases reflex lung contraction and reflex cardiac inhibition (Luckhardt and Carlson, 1921).

In four animals the brain was exposed dorsally and the rootlets forming the glossopharyngeal-vagus complex were isolated with a portion of the brain. Upon electrical or slight mechanical (traction) stimulation motor effects were observed in one animal in both the stomach and the gills. The motor response in the stomach appeared either in the form of one or two weak contractions without inhibition or the contractions were followed by inhibition. However, in the rest of the animals so studied inhibitory effects only were observed on the stomach while motor effects occurred in the gills. This would possibly suggest the presence of a few motor fibers in the vagus for the stomach of necturus but it must be granted that if such motor fibers do actually exist, they must be extremely in the minority. In other words, the predominance of the inhibitory fibers in the vagi is apparently in inverse order to the arrangement of these fibers as found in the same nerve in other animals. In a few experiments an attempt was made to stimulate the splanchnic nerve fibers supplying the stomach after laparotomy. Rapidly induced electric shocks were used. Out of four such animals three gave positive and one negative results. In those stomachs in which peristaltic activity was exhibited the contractions started in the upper third and advanced slowly toward the pylorus but none of the waves were observed to pass over the pylorus. In two animals the peristaltic contractions resulting from the stimulation were so strong at times as to obliterate completely the lumen of the stomach. In the other animal the contractions were weak. Several contractions may be obtained from the same stomach by repeating the stimuli, but the contractions gradually become weaker until finally they cease altogether. Therefore, the experimental evidence for the existence of motor excitatory fibers via splanchnics to the stomach is much more pronounced than for the presence of such fibers via vagi in necturus.

The results here presented on the inhibitory influence of the vagi on the stomach of necturus together with certain inhibitory phenomona presented in the literature on the esophagus of the turtle, the hearts of fishes and the lungs of frogs, necturi and other salamanders indicate that

this inhibitory innervation via vagus plays an essential rôle in the motor activity of the gastric mechanism. This gastric inhibitory action probably does not arise through the action of sympathetic fibers that might join the vagal trunk, due to the fact that stimulation of the vagus invariably produces inhibition which would tend to rule out this factor as being of any importance. However, the experimental results in toto on one species cannot be transferred to another species, because of the evident variations in the degree of the primitive motor control retained in the anterior portion of the gut in different animal groups.

CONCLUSIONS

1. The contractions of the empty stomach of *Necturus maculatus* are practically continuous like those of the bullfrog but the individual contractions are weaker and the intervals of rest are much longer. No changes in gastric tonus were observed.

2. Weak acid or alkali when introduced directly into the empty stomach produces temporary inhibition. Body movements of the animal also cause a similar effect.

3. Bilateral vagotomy after a prolonged period of gastric inhibition leads to a marked augmentation of the normal contractions by removing the inhibitory influence on the stomach.

4. Electrical stimulation of the vagus or even slight traction on either the sectioned or the unsectioned nerve when the stomach is exhibiting motor activity results in almost an immediate inhibition of its movements.

5. Stimulation of the central end of a vagus after unilateral vagotomy either with induced electric shocks or by traction causes an inhibition of the movements of the empty stomach reflexly, the nerve pathway being via intact vagus nerve since bilateral vagotomy abolishes the reflex. It is also possible to obtain a similar reflex on the stomach from stimulation of the gills with the vagi intact.

6. The inhibitory influence of the vagi on the stomach of *Necturus* is predominantly, if not exclusively, inhibitory, and it therefore plays an essential rôle in the motor activity of the gastric mechanism. It is also probable that the splanchnic nerves supply motor excitatory fibers to the stomach.

I desire to express my gratitude to Dr. A. J. Carlson for his helpful suggestions and criticisms in carrying out this work.

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STUDIES ON THE DUAL INNERVATION OF THE DIAPHRAGM WITH SPECIAL REFERENCE TO TONUS AND NUTRITION

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For the last ten years I have carried out experimental investigation of the autonomic innervation of voluntary muscle. From the results I came to the conclusion that the nutrition and tonus of voluntary muscle were controlled by the cerebro-spinal, extrapyramidal innervation and also by the sympathetic and parasympathetic innervations. The essence of these studies will be reported under several headings.

STUDIES ON THE TONUS AND NUTRITION OF THE DIAPHRAGM. The diaphragm gets its nervous supply mainly from the phrenic nerve. Besides this, it has the sympathetic innervation coming from the celiac ganglion. According to Aoyagi, Felix and others, the phrenic nerve contains a large number of non-medullated fibers derived from the cervical sympathetic chain. As these non-medullated fibers are entirely eradicated by extirpating the cervical sympathetic, especially the stellate ganglion, it is seen that we can completely do away with the sympathetic system without impairing the somatic innervation if we extirpate the stellate ganglion and the abdominal sympathetic. On the other hand, the cerebro-spinal innervation can be removed by severing the roots of the phrenic nerve. For these reasons the diaphragm is the handiest muscle, controllable at will, for studying dual innervation. Therefore we utilized the diaphragm to the utmost for our investigation. The unsatisfactory part of this study is that of the parasympathetic innervation, of which nothing is known.

Investigation of the tonus of the diaphragm by means of roentgen rays. This work was done in coöperation with Hiramatu, Takagi and Konisi. The normal roentgenologic appearance of the diaphragm of the monkey was first fully studied; then the nervous control of the diaphragm was removed and the changes that followed were examined. The diaphragm, when its tonicity is decreased, is drawn up by the negative pressure of the thoracic cavity, and a condition develops clinically called relaxatio or eventratio diaphragmatica. As the formation of the relaxatio is prevented by the close proximity of the liver, a heavy organ, the animal for this experiment

must be one such as the monkey, whose liver is one-sided, and whose thorax is not smaller than the abdomen. Therefore we operated on the left side of the diaphragm of the monkey for our experimental observations.

When the left abdominal sympathetic was eradicated, the tonicity of the left half of the diaphragm was decreased, and that side of the diaphragm was a little elevated toward the thoracic cavity. This picture was made more distinct by filling the stomach with air, although later the elevation could not be demonstrated in this way.

In the second experiment the sympathetic element of the left phrenic nerve was removed by extirpating the left cervical sympathetic at the same time as the left abdominal sympathetic. Then the elevation of the left side of the diahragm appeared as in the previous experiment. However, this elevation disappeared later.

No change in the movement of the diaphragm was noticed in these two experiments.

In the next experiment, the cerebro-spinal nerve roots of the left phrenic nerve were cut off. As some of the roots of the phrenicus are derived from the subclavius, this was also carefully removed. The left side of the diaphragm immediately slackened and became elevated. The elevation of the diaphragm was most distinct two days after the operation, and the degree was about the same as in the cases in which the sympathetic control was eradicated. The decrease of tonicity of the diaphragm was not very pronounced nine days after the operation, but the movement of the left side of the diaphragm was permanently lost.

The entire evulsion of the left phrenic nerve was effected by plucking out both the cerebro-spinal and the sympathetic fibers to it. Then the left side of the diaphragm lost all its tonicity, and was elevated remarkably from a few days to about the two weeks after the operation. The relaxatio diaphragmatica was produced and the heart was seen to have moved to the right side of the chest. This decrease of tonicity of the diaphragm was permanent. By the extirpation of the abdominal sympathetic, the slackening became still more distinct.

It is evident from these results that both the cerebro-spinal roots and the sympathetic fibers participate in maintaining the tonicity of the diaphragm. The removal of either causes only temporary slight decrease of tonicity of the diaphragm. When both of them are eradicated, the decrease of the tonicity is marked and permanent. Therefore it seems to follow that deficiency of the cerebro-spinal innervation is compensated by the sympathetic innervation, and the tonic defect caused by the removal of the sympathetic innervation is compensated by the cerebro-spinal innervation. The parasympathetic innervation may also participate in this. When one factor is deficient, there is a compensatory increase in the other factor.

Chemical investigation of the tonicity of the diaphragm. This part of the experiment was conducted in coöperation with Maeda and Toyama. It was found by Pekelharing that the increase of creatin was parallel with the increase of tonicity of the voluntary muscle. In the following experiments, the methods employed in the experimental groups 4 and 5 were those of Baumann and in the others those of Pekelharing. In the control group, we determined the creatin content of both sides of the diaphragm of each of four dogs. The value for 1 gram of the muscle tissue of the diaphragm was from 3.05 to 3.85 mgm. The maximal difference in the values of creatin content of both sides was 0.06 mgm.; this amounted to only 2 per cent of the whole creatin content.

In experimental group 1 all the nervous fibers, both cerebro-spinal and sympathetic, leading to the left side of the diaphragm, were removed. The creatin content of both sides of the diaphragm was compared from one hour and ten minutes to twenty and a half hours after the operation. The creatin content of the left side showed an average decrease of 0.26 mgm., with the maximum 0.39 mgm. and the minimum 0.15 mgm. Hence even the minimal decrease amounted to more than twice as much as the maximal difference in the control group. The difference of creatin content in the two sides increased progressively after the operation. Thus the eradication of all the innervation of one side of the diaphragm causes a marked decrease in the creatin content of the same side.

In the roentgen-ray investigation, it was shown that both the cerebro-spinal and sympathetic fibers participated in the tonic function of the diaphragm. The next step in the investigation was to determine which tonus is mainly concerned with the creatin metabolism.

In experimental group 2 the sympathetic innervation only of the left side of the diaphragm was eradicated in six dogs. The creatin content of the left side of the diaphragm also showed a remarkable decrease after this operation. The value of the average decrease was 0.27 mgm. with 0.69 mgm. as maximum and 0.09 mgm. as minimum. The decrease of creatin was about the same as in the previous experiment.

In experimental group 3 the cerebro-spinal roots of the phrenic nerve were severed in sixteen dogs. All of those which were killed within ninety-five hours after the operation showed the increase of the creatin content in the side of operation. After a hundred sixteen hours, some of them showed an increase and the others a decrease. After more than two hundred thirty-eight hours, the creatin content decreased remarkably. Thus the creatin content increased in the side of operation for a certain period after the operation. The decrease of the creatin content shown in a certain period after the operation was probably due to extreme atrophy of the diaphragm in consequence of the operation. The decrease in the creatin contents of the muscles when they become atrophic was demon-

strated in another experiment. From the findings in twelve animals killed within ninety-five hours after the operation, it was clear that eradication of the cerebro-spinal innervation causes an increase of the creatin content. The minimal increase occurred fourteen and a half hours after the operation and was 0.005 mgm., the maximal increase (seventy hours after the operation) was 0.48 mgm., the average being 0.28 mgm.

In experimental group 4 all the innervation of the right side of the diaphragm was eradicated and the cerebro-spinal fibers of the left side severed. The right side of the diaphragm of such an animal, after thirty days, showed striking atrophy. The creatin content of the right side was 2.3 mgm. and that of the left side was 2.9 mgm., the difference being 0.6 mgm. The creatin content of the atrophic right side was thus remarkably decreased.

In experimental group 5 two dogs were used. The right cerebral cortex was destroyed and the creatin content of the diaphragm was determined. In the two cases, the creatin content was respectively 0.25 and 0.39 mgm. greater on the left side of the diaphragm than on the right side. That is, when one side of the cerebral cortex was destroyed, the opposite side of the diaphragm showed an increase in the creatin content.

These results may be summarized as follows: The eradication of all innervation, or of only sympathetic innervation, causes a decrease of the creatin content in the diaphragm. The eradication of the motor innervation, however, always brings forth an increase in the creatin content for a certain period after the operation. This phenomenon must be met with some satisfactory explanation. It was established in our previous experiments that the decrease of creatin content took place in the diaphragmatic muscles when both cerebro-spinal and sympathetic innervations were eradicated. Therefore the creatin increase in question must have something to do with the activity of the sympathetic innervation. It was shown in our experiment with monkeys that the compensatory increase of sympathetic innervation was responsible for the fact that after the eradication of the cerebro-spinal innervation the manifestations were only slight and the decrease in tonicity of the diaphragm gradually disappeared. The increase of creatin content in the diaphragm after the removal of the cerebro-spinal fibers, then, seems to be satisfactorily explained by this compensatory increase of the sympathetic innervation. Therefore it appears to be the sympathetic tonicity of all forms of muscle tonicity that is directly concerned with the creatin metabolism. As will be mentioned in a second report, an injury in the pyramidal tract causes increase in the sympathetic tonus. The creatin content increases also in the opposite side of the diaphragm when one side of the cerebral cortex is destroyed.

Bio-electrical investigation of the tonus of the diaphragm. As Dittler's electromyographic investigation on the tonus of the diaphragm was made

before the innervation of the diaphragm was understood, Fuzita of our laboratory took up the subject for investigation in the newly acquired light of the findings just recorded. Forty-two rabbits were used for experiments with the string galvanometer of the Cambridge model. The animal was fixed on its back and the upper part of the abdomen was so opened that the sternal portion of the diaphragm attached to the xyphoid process could be seen. The electrode was placed at the sternal portion of one side of the diaphragm. The string of the galvanometer measured 3 microns in diameter and the shadow of the movement of the string was magnified 400 times. It was so regulated that the excursion measured 10 mm. when 1 millivolt was introduced.

In the normal condition of respiration, the electromyograph shows a wavy picture. This curve is the result of the mechnical movement due to respiration. When the waves are high, that is, at the time of inspiration, a discontinuous-action current occurs and this is not seen at the time of expiration, as studied by Dittler.

The discontinuous-action current appears faintly but continuously when the animal is brought into the condition of apnea by forced respiration. If the forced respiration is interrupted, and the animal left in the condition of apnea, the wavy curve entirely disappears, and the discontinuous-action current appears continuously. As this discontinuous-action current is seen when there is no contraction of the diaphragm, it represents the normal tonus of the diaphragm. And because it is made weaker by the apnea, the discontinuous-action current seen at the time of normal respiration is the aggregate of that from the tonicity of the diaphragm, from its contraction (that is, the tetanic condition) and from the increase of the tonicity. As the discontinuous-action current entirely disappears at the end of expiration, the tonicity of the diaphragm must be considered as remarkably reduced at this stage. Immediately after the cervical sympathetic chain is extirpated, a change does not follow, but a few minutes later the excursion of respiration becomes great and at the same time the amplitude of the action current becomes remarkably large, sometimes twice normal. When the cervical sympathetics of ten rabbits, in the condition of forced respiration or in the condition of apnea, were extirpated, the amplitude of the discontinuous-action current also became large. When the vagus of one side was severed, the respiratory excursion became large after a few minutes, and the amplitude of the action current also increased. No change follows immediately after the injection of atropine (2 cc. of 1 per cent solution) but after a few minutes the amplitude of the action current increases. When the cerebro-spinal roots are severed, the oscillatory-action current entirely disappears. The tonicity of the diaphragm giving rise to the oscillatory-action current, therefore, disappears on the removal of cerebro-spinal innervation.

No effect was seen immediately after an injection of epinephrin (2 cc. of 1 per cent solution) and of pilocarpine (2 cc. of 1 per cent solution) although the amplitude of the action current increased after a few minutes. However, the oscillatory-action current does not appear after the injection of epinephrin or pilocarpine if the cerebro-spinal roots have previously been severed. Therefore it is the cerebro-spinal tonicity that accounts for the oscillatory-action current. This action current accompanying cerebro-spinal tonicity is augmented by the extirpation of the cervical sympathetic.

It is clear from the foregoing experiment that cerebro-spinal tonicity is compensatively accentuated after the extirpation of the cervical sympathetic. It is not known whether the vagus has anything to do with the tonicity of the diaphragm nor how it reaches the diaphragm. However, the section of the vagus and the anesthetizing of it by atropin increases the amplitude of the oscillatory-action current more or less, but not to such a degree as when the cervical sympathetic is removed. The discontinuous-action current is also increased by the administration of epinephrin and pilocarpine. As will be mentioned later, this is similar to such cases as those in which the increase in sympathetic and parasympathetic tonicity would cause increase in cerebro-spinal tonicity. The discontinuous-action current does not return when the sympathetic and parasympathetic tonicity are increased if the cerebro-spinal roots are severed. Therefore the sympathetic and parasympathetic tonicity even when increased does not seem to give rise to the discontinuous-action current.

Trophic innervation in the diaphragm. I have studied this subject in coöperation with Simbo. According to the view held in general, the nutrition of muscles is governed by the ganglion cells of the anterior horn of the spinal cord. If the cerebro-spinal fibers in the phrenic nerve are severed, the diaphragm naturally must fall into total atrophy. The diaphragms of five dogs in our experiment, after section of the cerebro-spinal fibers in the phrenic nerve, did not necessarily show, after two or three months, a remarkable change; there was only slight atrophy of the muscle fibers and nuclear hyperplasia. After a long time quite marked atrophy of muscle fibers appeared, the fibers became twisted and nuclear hyperplasia was also increased. However, this form of atrophy is not accompanied by degeneration. Even in the case of marked atrophy, the muscle fibers do not disappear so completely that all the tissues are replaced by connective tissue. The diaphragm of a monkey, one year and seven months after the operation, failed to manifest marked atrophy.

In the next experiment, the sympathetic fibers as well as the cerebro-spinal fibers in the phrenic nerve were severed; that is the whole phrenic nerve was evulsed. Not until two or three months after this operation did marked atrophy of the diaphragm develop. The muscle layer of the diaphragm became thin and yellowish. From four months to one year

after the operation, the atrophy became extreme and no muscle fiber could be recognized by the naked eye. Microscopically, marked atrophy and coiling up of muscle fibers, nuclear hyperplasia, and hyaline and fatty degeneration were seen. In an extreme case, the whole diaphragm was converted into a sheet of connective-tissue membrane. It is to be emphasized at this time that the atrophy and degeneration first appeared at the pars costalis, then at the pars sternalis; the pars lumbalis was the last to be affected. The change in the pars lumbalis is sometimes fairly marked but never so extreme that the muscle fibers are entirely lost; in the pars costalis, however, the change is extreme.

From the results obtained in this experiment, it would seem that the survival of the sympathetic fibers in the phrenic nerve prevents the progress of atrophy in the diaphragm. On the other hand, however, there are such facts to be considered as follow.

The younger the animals the more quickly and more completely does atrophy develop. While only a little change appeared in the diaphragms of the mature dogs sixty-five days after section of the phrenic nerve, the diaphragms of the young dogs were changed almost entirely into connective-tissue membranes sixty-two days after the operation. The extent of atrophy also must be considered in the light of the individual disposition.

Mindful of excluding these factors, we undertook another set of experiments in which we used two monkeys and sixteen dogs. On one side of the diaphragm the phrenic nerve was plucked out, and on the other side only the cerebro-spinal fibers in the phrenic were severed. The resultant atrophy was always much more remarkable in the former case than in the latter. From this it became clear that the sympathetic fibers play an important part in the nutrition of the diaphragm.

The relatively slight degree of the atrophic change in the pars lumbalis of the diaphragm on eradication of the phrenic nerve seemed to be due to the innervation of the pars lumbalis by the abdominal sympathetic. To establish this, the abdominal sympathetic of four dogs was removed, and the cerebro-spinal roots of the phrenic nerve of the same side were severed. The results showed that the atrophy of the pars lumbalis was strikingly greater than that of the pars costalis. Hence it was shown that the sympathetic innervation works against the muscular atrophy caused by obliteration of the cerebro-spinal innervation.

We went further to see what change the ablation of sympathetic innervation alone would bring forth. The abdominal sympathetic only was removed in one monkey and four dogs, and both the abdominal and cervical sympathetics of the same side were removed in three dogs. The animals in the latter case did not live long enough to be examined. In the former case, however, the diaphragm, especially its lumbar part, showed a striking change. A young dog presented, only about a month

after the operation, a high degree of muscular atrophy, and the diaphragm appeared thin and yellowish. Coiling up of muscular fibers, nuclear hyperplasia in the sarcolemma, and hyaline and waxy degeneration were seen. Yet, these forms of degeneration have been found not to be constant; it is rather unusual to find them after the obliteration of sympathetic innervation. It is noteworthy that some hypertrophic, rounded muscular fibers were found among the highly atrophic ones.

The changes in the diaphragm on the eradication of the sympathetic innervation might be attributed to the changes in the innervation of blood vessels. The removal of the sympathetic fibers, however, causes vascular dilatation, which should result in local hyperemia. This could not make the nutritional disturbance of muscles, caused by the elimination of the cerebro-spinal innervation, much worse. It is also inconceivable that the vascular changes should be especially marked on removal of both the sympathetic and cerebro-spinal as compared with the changes produced when the sympathetic innervation alone is destroyed. According to our studies on the innervation of the muscular tonus of the diaphragm, the effect of elimination of the sympathetic innervation alone is not distinct because of the compensatory increase of cerebro-spinal tonicity and vice versa. The trophic relation of muscles also appears to be analogous. If the tonic and the trophic innervation reach muscles by the same nerve fibers, the probability of this assumption is very great.

CONCLUSIONS

Tonic and trophic innervation of the diaphragm is of cerebro-spinal and autonomic origin. The two systems of innervation are inclined to compensate each other. The creatin metabolism of the muscle is related mainly to the sympathetic innervation.

STUDIES ON ADRENAL INSUFFICIENCY

IV. The Influence of Intravenous Injections of Ringer's Solution upon the Survival Period in Adrenalectomised Dogs

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These experiments were undertaken with the object of gaining information on the pathology of the condition which develops after removal of the adrenals and which culminates in a more or less characteristic train of symptoms described in detail in a previous paper (Rogoff and Stewart, 1926). Another purpose was to lengthen, if possible, the period of survival of adrenalectomised animals so that they could be studied, in various ways, for a longer time. All the experiments were performed on dogs. When we began the work, all statements in the literature greatly underestimated the survival period. Our control animals, i.e., those in which no treatment was adopted, constitute a series of more than 100 dogs that has been acquired over the period of years during which we have been engaged in studies on adrenal insufficiency.

It is absolutely necessary to have such a series, as the statements in the literature as to the survival period are vitiated by inadequate surgical technique. This is true not only for dogs but also for other animals. Any writer who compares his results in animals "treated" in any way, with for instance, the notoriously inaccurate results of Strehl and Weiss in dogs, cats, etc., is certain to be in error. This is illustrated by quite recent work.

In paper I of this series (Rogoff and Stewart, 1926) we have given data on the length of survival and the period of good health after adrenalectomy in 74 dogs that were not subjected to any treatment. In another article (paper V of this series), will be published additional data on 36 control animals. A very small number of the control dogs lived about a fortnight after removal of the second adrenal. The majority survived about a week. With this large number of controls for comparison it is not difficult to conclude with confidence whether the survival period

[1] A preliminary account of this investigation was published in Proc. Soc. Exper. Biol. and Med., 1925, xxii, 394.

has been influenced by any given treatment or not. Untreated dogs never survive the onset of the characteristic terminal symptoms more than a very few days. These symptoms suggest a severe and increasing intoxication. The nature of the toxic substance or substances is unknown, but it was thought that important suggestions might be derived if injection of a simple salt solution could be shown to exert a favorable action upon the symptoms and survival period. These experiments were begun in 1924, and continued through 1925 and the early part of 1926.

TABLE 1

Adrenalectomised dogs treated by intravenous injections of Ringer's solution

RECORD NUMBER	WEIGHT		INTERVAL BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION
	First operation	Second operation					Right	Left	Blood	Congestion	
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gram*	*gram*			
86–2			12	19	12	8			+		+
96–6	9.5	10.2	6	19	8	10	0.60	0.65	+−	+−	
97–8	11.7	12.9	27	33	5	16	0.62	0.56	+++	+++	+++
98–0	11.5	11.0	12	14	20	13½	0.65	0.54	++	++	++
100–2	11.6	13.3	13	32	17	26	0.78	0.90	++	++	++
100–5	8.1	8.3	12	15	5	11	0.45	0.45	+	+	++++
102–6	11.5	13.3	39	17	6	14½	0.75	0.85	+++	+++	+++
113–1	9.2	8.5	31	6	16	5	0.40	0.37	++	++	++++
113–3	10.2	9.3	31	5	12	5	0.57	0.66	0	+	+++
113–4	9.4	9.5	53	14	4	4½	0.58	0.48	+−	0	++
114–0	12.4	12.6	14	13	15	7	0.70	0.60	++	+	++
117–0	11.1	10.3	33	53	16	45	0.70	0.60	+−	0	++++
117–2	7.7	7.5	33	38	2	19	0.65	0.72	+	+	++++
118–3	11.2	10.8	18	20		19	0.55	0.60	++	+++	++++
118–4	6.8	7.5	30	20	3	15	0.65	0.80	++	++	+
119–0	10.7	10.8	23	3		3	0.73	0.85	+	++	++++
119–1	9.1	8.8	23	6	12	6	0.72	0.86	0	0	+++

All dogs are males except 100–2, 102–6, 113–4, 118–3 and 118–4. Right adrenal excised first in all the dogs except in 113–1, 113–3, 113–4 and 114–0.

In the large majority of our experiments the injections were given once daily, by intravenous route. Ringer's solution, having the following composition, was used—sodium chloride 9 grams, potassium chloride 0.42 gram, calcium chloride 0.24 gram and sodium bicarbonate 0.1 gram per 1000 cc. of water. To this was added, in most cases, 2 to 3 grams of dextrose per liter, in a number of cases 5 to 10 grams per liter. The dose administered was usually about 100 cc. per kilogram of body weight, injected during a half to three-quarters of an hour. The dose was purposely large in order to produce a decided effect. Free micturation

occurs immediately or soon after the injection. Evidence of increased intestinal peristalsis is present, and commonly defecation occurs. Occasionally there is vomiting.

Table 1 shows clearly that the period of survival, after the second adrenalectomy, is often much increased beyond the maximum period for control untreated dogs. Of the 17 animals included in the table more than half lived into the 18th day or longer. Three of these lived between 30 and 40 days and one survived for nearly 54 days. No such results were obtained in any of the large number of controls, very few reaching 14 or 15 days and the great majority dying in 7 to 10 days or less. This is conclusive evidence that life can be prolonged by these injections. As to the proportion of successful cases and the absolute prolongation of life, it is not to be supposed that we obtained optimum results. The dose was varied but little, except that the larger animals received a larger volume than the smaller. Variation in the amount or frequency of the injections might have given better results. One injection was nearly always given in the twenty-four hours. But when an animal was failing, several injections were sometimes given, with benefit. The rule was to start the treatment about 24 hours after removal of the second adrenal. We acquired the impression that it was advantageous to begin the treatment early. Still it may well be that a better result might have been obtained had the first injection been postponed longer than 24 hours after an operation so serious as adrenalectomy. It was not determined whether variations in the amount of dextrose affected the result. The work was too laborious and time-consuming to permit us to attempt more than the definite proof that the treatment exerted a marked influence on the survival period and the period of good health.

The immediate beneficial effects of the treatment often observed in animals already showing severe symptoms, including coma, are well illustrated in some of the protocols, e.g., that of animal 102–6, the protocol of which has already been published in paper III (Rogoff and Stewart, 1927). At that time our studies on the influence of pregnancy (and rut) on the survival period and the period of good health after adrenalectomy had not been made. It is scarcely possible to determine the relative importance of this factor and of the salt solution, and therefore animals 102–6 and 100–2 have been included both in table 1 of paper III and in table 1 of this paper. The protocol of 102–6 indicates that in this animal the injections were not only important but actually restored the animal when comatose and permitted its survival in good condition for a relatively long period, death occurring after $17\frac{1}{4}$ days. Animal 100–2 survived 32 days $16\frac{1}{2}$ hours. A description of a few typical experiments with condensed protocols will now be given.

Dog. Male. Record number 86-2. October 19, 1924. Right adrenal excised. November 10, left adrenal excised at 2:00 p.m. On November 11, in good condition; daily intravenous injections of Ringer were begun (1000 to 1400 cc., approximately 100 to 150 cc. per kgm. body weight with 2 grams dextrose per liter). November 13, reflexes exaggerated; tremor of leg muscles. Emesis after injection, later copious micturition. November 14, tremor of head and legs, relieved by the injection, which was followed by copious micturition and defecation (semi-liquid stools). November 14 to 18, condition very good; eating well and behaving normally. Temperature varied little from 38°C. No injection given on November 18 and 19. On November 19 total anorexia; has voided very little urine; lethargic and somewhat asthenic. Temperature 37.8°C. Restored to excellent condition by a Ringer-dextrose injection, which, as usual, was followed by diuresis and defecation. Weight 7.5 kgm.; lost 1 to 1½ kgm. since last operation, but in good health. Injections continued for remainder of survival period. November 20 and 21, condition good. November 22, some asthenia, muscular twitching; moderate anorexia. November 23, hallucinations. November 24, 9:00 a.m. Legs spastic, reflexes exaggerated. Hallucinations and short tonic spasm, followed by marked asthenia. At 11:30 a.m., injection followed by improvement. At 9:30 p.m. another injection with marked improvement. November 27, total anorexia for the past 3 days; asthenia recurring and relieved by injections. Today he regained moderate appetite. Condition unchanged till November 29, when asthenia became much more pronounced than heretofore. At 5:00 p.m., an injection was followed by temporary improvement. Died during night (November 29 to 30).

Dog. Male. Record number 96-6. November 18, 1924, right adrenal excised. November 24, left adrenal excised. November 25. Condition excellent; appetite voracious. Daily Ringer injections begun (about 100 cc. per kgm. body weight with 2 grams dextrose per liter). December 3, health excellent up to date. December 4, refused food; moderate asthenia. On December 5 and 6, asthenia was relieved by injections, but total anorexia persisted. December 7, has regained some appetite for certain foods (salmon, rabbit); asthenia no longer present. December 8 and 9, total anorexia, but no asthenia. December 10, total anorexia; marked asthenia; lethargy; cough; emesis (bile-stained material) and defecation (some tarry feces) about an hour after the Ringer injection. December 11, no change; some improvement following injection. December 12 and 13, very lethargic and asthenic. Restored to fairly good condition after injections. December 13, 10:00 p.m., comatose; died during the night.

Animal 86–2, which survived 19½ days was still in excellent condition on the eighth day after removal of the second adrenal, having received daily injections of Ringer-dextrose solution. On this day it was decided to omit the treatment. The following day the animal was worse. He became lethargic, moderately asthenic and developed total anorexia. But he was again restored to good condition by an injection of Ringer-dextrose solution and the daily injections, thereafter, continued to benefit the animal up till shortly before death.

This dog and animal 96–6, which survived 19⅓ days after removal of the second adrenal, afford good illustrations of the fact brought out clearly in table 1, that the interval between the first refusal of food and

the death of the animal may be much greater in the animals treated by intravenous administration of salt solution than in control, untreated animals. Thus animal 86–2 refused food 8 days and animal 96–6 refused it 10 days after removal of the second adrenal, although life was still prolonged for 11 and 9 days respectively. In a number of the animals anorexia and other symptoms were recovered from, repeatedly, under the influence of the injections.

In animal 97–8, which survived more than 33 days, there was total anorexia for the first 5 days after the second adrenalectomy. On the third day he developed coma with convulsions. The immediate beneficial influence of Ringer injection (which was given three times on this day) was only temporary, but the ultimate result was very striking. The following morning we found the animal walking about and normal in appearance and behavior. In control, untreated adrenalectomised animals the occurrence of coma with convulsions presages death within a relatively short time. Appetite appeared on the fifth day and continued good till the sixteenth day. Then food was refused, and although some appetite was regained it remained capricious and not nearly equal to normal. For the last 5 or 6 days of life there was total anorexia.

Dog. Male. Record number 97-8. Right adrenal excised December 18, 1924. Left adrenal excised January 14, 1925, at 2:30 p.m. Weight 12.85 kgm. January 15, at 10 to 10:30 a.m., Ringer 750 cc., with 2.5 grams dextrose. Urinated copiously; emesis about 15 minutes after injection, and again later. Total anorexia. January 16, total anorexia but seems strong; 10:30 a.m., temperature 39.3°C., pulse 152 (regular). Ringer 750 cc. with 2 grams dextrose. Urinated and defecated.

January 17, seems weaker; small stitch infection. 2:15 p.m., 750 cc. Ringer with 3 grams dextrose. Urinated small amount. 5:30 p.m., tetanic convulsion and deep coma; Ringer 1250 cc. with 3 grams dextrose. Urinated on table and afterwards seemed much improved, but lethargic. 9:00 p.m., tetanic convulsion and deep coma; Ringer 1000 cc. with 15 grams dextrose. Urinated, but became only slightly more conscious during injection. At 9:30 p.m., deep coma.

January 18. In the morning he was standing up and wagging his tail. 3:00 p.m., condition much improved; Ringer 1500 cc. with 5 grams dextrose. Urinated. Lethargic after injection. January 19, much improved. Took food. Growled at other dogs. 5:00 p.m., 1400 cc. Ringer with 3 grams dextrose. Copious urination.

Till January 28, he remained in good condition, eating well. Received daily, 1000 cc. Ringer with 2 to 3 grams dextrose. As on previous occasions he was lethargic for an hour or two after the injection. Temperature 38.8°C. to 39.1°C. Stitch wound healed.

January 29. Appetite less than usual; less alert. January 30. Very weak and somnolent; ate nothing. Not much urine voided. Temperature 38.4°C., pulse 88 (good). Weak in hind quarters. January 31, somewhat better. February 1, much improved; urinated well; fair appetite. Runs about the room as usual. February 2 to 4, fairly active; some appetite but not as good as a week ago. Weight 10.85 kgm. February 5, eating better, and February 6 worse. Same on February 7; weak in hind legs. On February 8, ate some salmon. February 9, ate rabbit; ran about as usual. Same on February 10. No marked asthenia. February 11, took a little food but vomited it up.

February 12, complete anorexia; gradually developing asthenia. When excited (by presence of a stranger), the hair on back and tail became erect and he barked violently and ran around. Apart from this he was more listless than usual today. Same on February 13. Took a run in the hall. Refused food. Temperature 38.2°C., pulse 100, respiration 27. February 14 and 15, not much change; some vomiting. Ringer (1000 cc. with 3 to 6 grams, once 8 grams, dextrose) was given daily from January 28 onwards. Died at 7:00 p.m. on February 16, 1925.

Similar observations were made in other animals, including 117–0 and 117–2.

Dog. Male. Record number 117-0. January 6, 1926, weight 11.1 kgm., excised right adrenal. February 8, 1926, weight 10.3 kgm., excised left adrenal at 2:00 p.m. February 9, excellent condition. At 3:30 p.m., 850 cc. Ringer with 6 grams dextrose. Urinated copiously. February 10 to March 8, received 1000 cc. Ringer with 6 to 8 grams dextrose daily. Remained in excellent health. Pugnacious as usual. Large appetite. Always urinated largely after injection. Occasionally, emesis of food taken soon after injection. Ate it up again.

March 9, slightly less active. Ringer 1000 cc. with 8 grams dextrose. March 10, weight 9.7 kgm. Emesis in morning (very green acid liquid). 12:00 m., ate well. 5:00 p.m., 1000 cc. Ringer with 8 grams dextrose. Some stretching, but condition good. March 11, unchanged. March 12, still eating, no asthenia, but beginning apathy. Injection continued daily. March 13 to 21, eating well; health good; weight 9.5 kgm. Ringer 1000 cc. with 10 grams dextrose daily. Emesis once or twice (some bile). March 21 and 22, appetite good. Tried to copulate several times.

March 23. Slow in eating meal (meat) but in time finished it. Ringer 1000 cc. with 10 grams dextrose. Good micturition; some emesis. Heart very slow and irregular before injection, which improved it. March 24. Apathy more marked. Ate meat. Green stool. Straddles slightly in walking. Some diarrhea after injection. No noticeable asthenia. March 25, fair condition but refused food. Diarrhea. Apathetic. Not asthenic. Somewhat improved after Ringer (1000 cc. with 10 grams dextrose). March 26, weight 9.2 kgm. Heart about 36 a minute, irregular. Greenish stools with very offensive odor. Ate only a little meat. March 27, after injection the heart rate went up from 36 to 140 to 150 a minute, but he did not micturate for 2 hours after injection. Took no food. Emesis (bile-containing, acid liquid). March 28. Quite weak. Heart irregular and slow, not improved by injection, although he walked better. March 29 to 31. Ringer continued (1000 cc. with 12 grams dextrose), and considerable improvement occurred. He ate some meat and rabbit several times and with good appetite. Walked much better. April 1, refused meat (rabbit). Urinated copiously during injection (1000 cc. Ringer with 15 grams dextrose) and afterwards. April 2. Poor condition. Some emesis. Weak on legs. Improved by injection. Urinated well. Walked. April 3, died, probably about 6:00 a.m.

Autopsy. Pancreas much congested. Stomach distended with bile-containing fluid. Practically no congestion. Same for duodenum, jejunum, ileum and colon. Rectum contained a small amount of blood-stained feces. Bladder distended with urine.

Dog. Male. Record number 117-2. January 6, 1926, right adrenal excised. February 8, 1926, excised left adrenal at 2:30 p.m. From February 9 onwards, daily injections were given (Ringer 650 cc. to 800 cc., with 6 to 8 grams dextrose). Health

good till February 19, with the exception of emesis (bile-containing material) on February 17, on which day the animal was somewhat lethargic and did not have much appetite. No asthenia. From February 20 to March 4, condition was very good most of the time. At intervals there was total or partial loss of appetite, sometimes emesis (bilious) and he slept more than usual. But always his condition returned to normal. On March 5 to 7, apathetic with loss of appetite, increasing to total anorexia. No asthenia. March 8, appetite fair, otherwise unchanged. March 8 to 16, little change. At times apathetic and lethargic; some loss of appetite with occasional emesis. No asthenia. March 17, slight asthenia (in hind quarters), improved after injection. No appetite. March 18, asthenia increased. Total anorexia. Coma with a convulsion; improved after injection. March 19, died early in the morning.

The continued survival, in good condition, of the animals in spite of anorexia, even when total, seems to us to be a suggestive point in the action of the Ringer-dextrose solution. Anorexia, it seems, need not be accompanied by the other serious symptoms which in the control animals presage death. That it can appear long before the fatal termination supports the view that it is only one of a number of symptoms indicating the profound derangement ultimately produced by adrenal insufficiency. The manner in which anorexia is modified by the injections explains the, at first sight, puzzling variations in the column in table 1, headed "Began to refuse food." Only the first definite refusal of food is noted there.

We have already expressed the opinion that animals dying from adrenal insufficiency never die of lack of nutriment. This is true even when the period of anorexia is prolonged to several or many days under the influence of injected salt solutions. The treatment, in such cases, dissociates the anorexia from the group of fatal symptoms. The loss of appetite is present with the accompanying changes, whatever they may be, but that does not suffice to cause death. There is no reason to believe that the small part of the caloric requirement covered by the injected dextrose made any essential difference in the survival period. No beneficial effects were observed in a small number of animals that were treated by subcutaneous administration of dextrose (5 to 10 grams dissolved in a small volume of salt solution, administered daily).

The modification of the symptomatology, for example the postponement of anorexia or recovery of appetite after total anorexia has appeared, is just as important a proof of the beneficial influence of the injections as the marked prolongation of life. The pathological appearances in the alimentary canal, although apparently not in the pancreas, are also mitigated by the injections as can be seen by comparing table 1 of this paper with tables 1 and 2 of paper I.

In our preliminary paper (Stewart and Rogoff, 1925) something has been said of possible ways in which the injections may be beneficial. We discussed especially the possibility that a poison or poisons accumulating in the absence of the adrenals may be washed out of the tissues (or perhaps

neutralised) by the thorough irrigation caused by the large injections of Ringer's solution. If the injections are delayed till the concentration of the blood described in a previous paper (Stewart, 1926) has occurred, the mere dilution may be expected to aid the circulation and thus produce a beneficial effect.

If it were practicable to wash out all the poison it is conceivable that animals would survive indefinitely. In our experiments it may be assumed to accumulate little by little, anchoring itself perhaps particularly in the nervous system, and also in the mucosa of the stomach and intestines which may be crippled at last and cease to eliminate it. That the mucosa is shielded in some way through the injections, seems to follow from the way in which anorexia and gastro-intestinal symptoms generally are staved off. An additional support to this view may be derived from the fact that the hemorrhagic condition observed *post mortem* in the alimentary canal seems to be less common and less severe than in the controls. This can be seen by comparing table 1 of this paper with tables 1 and 2 of paper I. The difference can hardly be explained as due to the washing out of blood from the vessels of the mucosa by the injected solution. For the congestion of the pancreas is usually as marked in animals which have received injections as in the untreated controls. In a few experiments we administered large quantities of water by stomach tube and in others repeated gastric and colonic lavage was performed. No beneficial effects resulted from treatment of adrenalectomised dogs by these methods.

What part, if any, the kidneys take in the elimination of the hypothetical poison cannot be stated. No definite lesion has hitherto been discovered in this organ in dogs. That the absence or delay of micturition after an injection is of sinister significance is true, but this may be due to circulatory deficiencies and not to any pathological change in the kidneys. It must be borne in mind that the first injection was made approximately 24 hours after the serious operation of adrenalectomy. Yet it is surprising how easily the large injections are handled by the circulation. The output of the heart is, of course, much increased, but we never saw an instance in which breakdown occurred, due to dilatation of the organ, during or immediately following an injection.

In the great majority of the experiments, we saw either temporary amelioration of the condition of the animal or survival for a period decidedly beyond anything observed in untreated controls. But occasionally animals were encountered which died with acute symptoms in a few hours after a single injection, given according to the usual routine, about 24 hours after removal of the second adrenal. In some of these it was thought that the Ringer's solution, kept for some time, might have been at fault. But fresh samples made with water from different sources, behaved in the same way. Different samples of dextrose were also investigated, but without

any definite result. It is possible that a more extensive investigation than we could afford time for would reveal the cause of the apparent grouping of some of these exceptional results. That it was not due to a difference in the injection liquid is indicated by the fact that some animals treated with the same solution showed the usual behavior, increased survival period with mitigation of symptoms, while at the same time others died in a few hours. The fact that some of these animals did not micturate during or after the injection indicates that the greatly increased volume of liquid was not being properly handled from the beginning, although the fatal symptoms might not appear for some time. Whether dilatation of the heart was responsible was not determined. A breakdown of the renal mechanism is a possibility. It is unknown whether excretion of liquid into the gastro-intestinal tract took place, although it is to be supposed that it did, since defecation (frequently diarrhea) after the injection usually occurred as in the other dogs. In some also there was emesis (bilious). Of about half a dozen animals, constituting the exceptional group, three were somewhat out of sorts at the time of, or immediately after, the second operation (cough; in one instance with nasal discharge). Three were apparently in normal health. A protocol from animal 115–3 will illustrate the course of events in this group.

Dog. Male. Record number 115-3. December 7, 1925. Weight 8.3 kgm.; right adrenal excised. December 16. Weight 8.1 kgm. At 1:45 p.m., left adrenal excised. December 17, condition excellent; pugnacious; good appetite. At 3:00 p.m., Ringer 750 cc. with 7.5 grams dextrose. Urinated and defecated (soft, yellow stool). At 3:30 p.m., urinated; emesis. At 4:00 p.m., lying down, apathetic. At 5:30 p.m., emesis had occurred (frothy mucus). Unsteady gait. Semi-stupor. Hallucinations; ran about in cage, aimlessly. At 7:30 p.m., increasing coma and convulsions. Died at 7:40 p.m. *Autopsy* at once. Thorax normal. Heart contracted. Some liquid in abdominal cavity. Practically no congestion in gastro-intestinal mucosa except in rectum. No blood. Pancreas much congested. Kidneys congested.

One dog (114–2), which died $3\frac{2}{3}$ hours after a single Ringer injection had a considerable amount of blood in the peritoneal cavity, probably from bleeding at the site of the second adrenal operation. Although it is likely that the loss of blood was not an important factor in causing death, the animal was not included in the group. It is possible that with the increase in blood pressure due to the injection, a ligature may have slipped. At the time of injection the dog seemed in very good condition. It took food (bread and milk) 10 minutes after the injection, but there was emesis later (bilious). The animal received somewhat less than the usual amount of solution (60 cc. per kgm. with 8 grams dextrose). There was practically no congestion of viscera except the pancreas, which was markedly congested. In two of the dogs, apparently in good health at the time of the injection, edema of the lungs was found *post mortem*. This and the in-

stance of liquid in the abdominal cavity, already mentioned, we consider evidence that the injection was not handled normally by the tissues.

Some control animals were used to test the question whether daily injections of such quantities of Ringer-dextrose solution as were employed in the adrenalectomised animals had any effect. No effect was observed in normal animals, except, of course, micturition. In one dog, afterwards adrenalectomised (98–0, table 1, paper I), the animal received, daily, an injection of from 1200 cc. to 1750 cc. Ringer (100 to 150 cc. per kilo of body weight) with 2 to 3 grams dextrose, for 10 days, without harm. The animal was then adrenalectomised in two stages in the usual way and used in the control series of adrenalectomised dogs. Another dog (115–1, table 1, paper I) after removal of the right adrenal, received daily injections of Ringer (750 to 850 cc. (100 cc. per kgm.) with 5 to 10 grams dextrose) daily for 8 days, beginning with the day after the adrenalectomy. There was occasional emesis and diarrhea; the appetite varied, although total anorexia was not seen. As some respiratory symptoms were present, after the injections, these were discontinued. The respiratory symptoms disappeared after about a week except some nasal discharge, and the second adrenal was removed. The experiment furnished no evidence that the injections, begun at so early a period after removal of one adrenal, had any harmful effects, unless they might have contributed something to the respiratory trouble.

While we are unable at present to explain a small group of exceptional results (3 animals apparently in good health when the injection was given) we do not consider that they constitute a contraindication for the therapeutic use of the procedure in certain emergencies, in patients. The dose, in proportion to body weight, would be much smaller than was given to the dogs. It would probably be better to repeat the injection at shorter intervals than to give a very large quantity at one time. In some respects the conditions would seem to be more favorable in human cases than in the animals. No operation would have preceded the injection, and some cortical tissue would generally be present and functioning. Let it be repeated, injection of salt solutions could be contemplated only in emergencies, since they could not "substitute" for a missing hormone. It may be, however, that when cortical extracts were being administered an injection of salt solution might act as an adjuvant when symptoms suggested that the extracts were not exerting a sufficient "substitute" action to prevent toxic effects of suprarenal insufficiency. The accumulated poisons once "washed out," the extracts might be sufficient for a further period.

SUMMARY

Marked prolongation of the survival period and of the period of good health was caused in adrenalectomised dogs, by daily intravenous injection of Ringer's solution (generally about 100 cc. per kilo of body weight). Dextrose was almost always added. A table is given showing results on 17 animals. One lived 13 days, 15 hours; one 14 days, 4 hours; one 14 days 20 hours; one 15 days, 5 hours; one 17 days, 6 hours; one 19 days, 8 hours; one 19 days, 12 hours; one 20 days; one 20 days, 3 hours; one 32 days, 17 hours; one 33 days, 5 hours; one 38 days, 2 hours; one 53 days, 16 hours.

Marked amelioration of symptoms, even when acute, is almost always produced during and immediately following injection. Sometimes animals have been rescued, when already comatose, and have lived a long time in good health. Possible ways in which the injections may act are discussed in the paper.

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STUDIES ON ADRENAL INSUFFICIENCY IN DOGS

V. The Influence of Adrenal Extracts on the Survival Period of Adrenalectomised Dogs[1]

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As soon as we had accumulated a sufficient number of control experiments on animals adrenalectomised but not treated in any manner, we were in a position to test the influence of various measures upon the symptomatology and particularly upon the duration of life and of the period of good health. These, especially the total period of survival after removal of the second adrenal, are definite numbers expressed in days and hours, which can be compared without guesswork with the control tables. At this point it may be well to introduce a few remarks which apply to the comparison of results under imposed conditions (treatments, pregnancy, heat, etc.) with the controls. We believe that the points enumerated must all be taken into consideration if correct conclusions are to be drawn.

In order that the comparison may have any value it is essential that the surgical technique, a difficult one, should be improved to the point at which few, if any, animals die within three to five days of the second adrenalectomy. When the results on animals operated upon so poorly that the fatal result (within a day or two) in most or all of them has been determined by the operation as such the so-called controls can only mislead. Especially is this the case where the writer does not even assemble a sufficient number of controls of his own but relies upon the utterly inadequate data presented in the older literature and in current monographs. Experiments in which death can be legitimately ascribed to adrenal insufficiency can alone be utilised. It is never permissible for an experimenter with an inadequate technique to assume that his operations will be of a uniform degree of badness in a control set of animals and in a set subjected to a certain procedure, the effect of which is to be tested, so that this factor can be considered as eliminating itself. It is only by improving the technique to the point at which the operations are uniformly well done that useful comparisons can be instituted.

[1] A preliminary account of the experiments was published in Science, October 7, 1927, lxvi, no. 1710, 327.

This being given, it is seldom possible to compare averages of the survival periods of two sets of results. Where the number of results is small and the individual variations large comparison of averages can only lead to error, as is too well illustrated in literature.

At present no evidence can be so unequivocal as an increase in the survival period because of its definite arithmetical nature. In all our comparisons of treated animals or of animals in various physiological conditions (pregnancy, heat) with controls we have considered it indispensable to drawing a positive conclusion that this test should be positive.

It is obvious that long survival attributed to treatment or other causes may be due merely to incomplete removal of the adrenal tissue. The literature is strewn with instances of this error, not always sufficiently considered by the investigators, not always discovered by them but undoubtedly present. In none of more than 200 dogs operated on by us has any adrenal remnant ever been found. All the adrenalectomies were total. Careful exposure of the gland at operation is quite important. When the gland is properly excised and the site of the operation inspected any remnant can be easily detected and removed. Also it can be seen that the excised gland is intact. Nevertheless, most careful search is always made *post mortem* for so-called adrenal "rests" or accessories. In one dog a portion of an adrenal separated from the rest by a constriction was seen and removed at the operation. In another dog a small accessory adrenal was seen and removed at operation.

A necessary precaution in a large series of animals subjected to a given treatment is to guard against the possibility that an apparently positive result may be due to increasing facility in the technique. Therefore, a series of controls obtained early in the investigation should not be relied upon exclusively but a new control series should be made while the "treatment" observations are going on. We have done this in 36 additional animals without finding any material change in the results, which are displayed in table 2. Additional controls may sometimes be obtained by using a treatment series, which has clearly yielded a negative result, as a control series. This expedient can only be adopted with great care. As regards the necessary number of animals in a series, no definite rule can, of course, be laid down, but an inconsiderable number such as six or seven, is of little use, unless the results are almost uniformly and strikingly positive.

One more point deserves mention, although it will readily occur to anybody who gives even a moderate degree of attention to the matter. None of the results obtained by treatment in our observations can be considered optimum. For example, with the Ringer's solution injection the amount of labor involved was far too great, as pointed out in the paper on that subject (paper IV), to permit sufficiently large series to be as-

sembled with varying doses, varying intervals between the injections and varying composition of the solution. All that could be done by us was to adopt an arbitrary dose of one and the same solution injected always at approximately the same interval of time. The same was true of the injections of adrenal extracts. It can therefore be confidently assumed that our results, although quite strikingly positive, are not the best which can be looked for.

This paper is devoted principally to the question whether evidence is obtainable that the adrenal (cortex) contains a substance which after extraction can be introduced into animals deprived of their adrenals and can prolong life and the period of good health beyond the maximum values in the control series. It will be seen when table 1 and some typical protocols are studied that the answer to this question is unequivocal; a not inconsiderable number of the treated animals do survive far beyond anything seen in untreated controls. The period of good health appears to be correspondingly prolonged, perhaps relatively even more than the total survival period. Not seldom the animal continues to eat almost up to the end, so that it may be impossible to notice the onset of anorexia as a definite event presaging death and preceding it. This is usual in the controls some days before death. It must be repeated that at present we do not deduce a positive conclusion from a *modification* of the symptomatology, although we believe this may be observed, unless the total survival period is increased without question beyond the limits seen in the controls. We have also gained the impression that the pathological changes in the gastrointestinal mucosa are modified by treatment with extracts, hemorrhagic congestion in the mucosa being less common and less extensive. The congestion of the pancreas, however, is not affected. We have already (Stewart and Rogoff, 1925; Rogoff and Stewart, 1926) developed a hypothesis of the significance of the morbid changes in the alimentary canal and have found some evidence in support of that hypothesis in the modification of the changes by Ringer's solution injections. Whatever their relation to the lack of adrenal function, it is significant that the symptoms and pathology should be capable of being modified by treatment. This is the case even in animals which do not outlive controls. As already pointed out (Rogoff and Stewart, 1927) the power to keep the treated animals alive, decidedly beyond the maximum period for controls, is a very severe test since every indispensable factor which the adrenals supply to healthy existence must be substituted for by the gland extracts, whereas the substitution of only one factor might suffice for the prevention of an important but not necessarily in itself a fatal symptom, such as anorexia.

A little more attention may be profitably given to table 1. It contains 29 animals, 20 males and 9 females, non-pregnant and, so far as could be determined by careful observation, not in heat. Since we have shown that

these conditions tend to prolong life beyond the limits seen in the controls, males alone are employed as far as possible.

TABLE 1

Animals treated with extracts of dogs' adrenals

NUMBER OF ANIMAL	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION	NUMBER OF EXTRACT
	First operation	Second operation					Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*				
123–0	9.0	9.0	12	13	13	13	++	++	+++	II
123–1	7.4	7.7	13	21⅔			0	++	+++	II, V
123–3	10.0	9.7	13	17⅔		17	0	0	++++	II, V
123–6	9.0	8.8	15	9	6	9	+++	++	++	V
123–7	9.6	9.2	22	10¾		9	++	++	++++	V
123–9	6.5	6.2	24	6	21½	5	++	+++	+++	V
124–1	10.6	10.4	21	78⅔		76½	0	0	0	V, XII
124–2	10.6	9.7	24	6	22	5	++	++	++	VI
124–5	8.5	9.4	69	12	2	11	0	++	++++	XV
124–7	9.5	9.6	64	9	4	8	++++	+++	+++	XV
124–9	9.3	9.5	12	10½		10	++	++	+++	XII
125–2	8.6	8.4	11	7½		6½	+	+	++++	XII
125–3	7.8	7.3	13	5⅔			+−	+−	+	XVI
125–5	10.0	9.6	35	10	23	9½	+++	+++	++++	XIII
125–7	7.0	7.2	38	27⅔		27	0	+−	+++	XVI
126–1	13.5	12.2	41	8	21¼		++++	++++	+	XIV
126–6	10.8	10.2	42	9⅔		8	++	++	+++	XV
126–7	11.1	10.2	19	7	1½	6	+++	++++	++++	XIV
126–8	10.9	10.2	20	12⅔		11	+++	+++	++++	XV
126–9	9.7	9.3	20	12	½	11	0	+	++++	XV
127–3	12.1	11.3	14	11	4	9½	+++	+++	+++	XVII
127–4	8.9	8.1	15	7⅔		7	+++	+++	+++	XII*
127–6	10.9	10.2	17	15	22	15	++	++	+++	XVIII
127–9	10.8	10.2	19	19¾		19	+++	++	++++	XIX
128–0	11.8	11.3	18	22⅔		21½	++	++	++++	XIX
128–3	7.8	7.4	13	9½		7	+++	++	++++	XIX
129–1	15.3	15.5	16	11⅔		10½	+++	+++	++	XXI
129–3	14.0	14.6	14	7⅔		4	+++	+++	+++	XXI
129–7	11.8	11.4	13	5	20	4	++++	+++	+++	XXI

All the dogs were males except 123–6, 123–9, 124–2, 124–5, 124–7, 125–3, 125–7, 126–6 and 127–6.

* Old material.

The occasional rapid improvement after extract injections with the restoration of the animal to a further period of health is sometimes observed although usually less striking than in the series treated by Ringer's solution.

TABLE 2

Additional control adrenalectomised dogs

NUMBER OF ANIMAL	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION
	First operation	Second operation					Blood	Congestion	
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*			
125–4	9.1	9.1	11	11¾		10	+++	+++	++++
125–6	5.8	6.5	23	8½		7	++	+	+
125–8	7.4	7.3	41	8½		6			
125–9	9.2	8.3	53	15¾		14	++++	++++	++++
126–0	11.1	9.7	53	6½		5	+++	+++	++++
121–1	7.9	7.8	14	11	5½	10	0	0	++
126–2*	11.7	9.3	56	5	4½	4	++	++	+++
126–3	10.0	10.0	40	12½		11	++	++	+++
127–0	11.3	11.2	20	9	7	8	+++	++	++++
127–7	10.9	8.5	42	12½		10	0	0	+++
128–2	12.2	11.7	15	14¾		13	0	+	+
128–9	9.1	9.8	37	15	17	12	++	+++	+
129–4	8.6	10.6	35	4	20	4½	+	+	+++
129–8	9.4	10.8	29	11	23	10	+++	+++	+++
129–9	7.0	7.5	50	8	22	7	+	0	++
130–0	9.6	9.9	32	6	4½	5	++	++	++++
130–1	7.1	9.6	55	8	23	7	++++	++++	++++
130–2	6.9	8.7	55	9½		7	++++	++++	++++
130–3	7.2	7.9	16	8¾		7	+++	+++	++
130–4	6.3	7.0	30	6¾		4	0	+	+++
130–8	6.2	6.1	19	6½		3–4	0	+	++
130–9	11.4	12.1	21	15	17¾	13	+++	+++	++
131–0	9.9	10.4	21	11			0	0	+++
131–1†	9.3	9.9	16	4	20	3	0	0	++
131–3	13.0	13.0	16	13½		12	+	+	++++
121–3	9.1	11.0	32	4	2½	3	++	++	++++
121–4	9.5	8.2	18	5	9	4	+	++	++++
121–5	5.7	5.8	15	11	15	9	+++	++	++++
121–6	7.5	8.2	30	12	16	10		+	+++
122–2	9.3	9.2	21	12	6½	11	++++	++++	++++
122–5	9.5	9.2	15	12	4		+++	+	++++
123–2	6.7	6.4	13	5	8	4		++	++++
123–4	8.7	8.1	13	7	10½	5	+++	+++	++
129–0	13.1	13.1	20	9½		8	+++	+	+++
131–4	14.5	14.0	15	6⅞		5	++	+++	++++
131–6	7.5	8.1	15	9	3½	7	+++	+++	+++

* Had weakness and tremors in muscles of legs and neck before and after operations.

† Very mangy before and after operations.

These effects are not to be neglected in deciding whether an extract is potent or not. Their occurrence will be illustrated in some of the protocols reproduced.

A more detailed examination of table 1 is now necessary. The 29 animals therein contained represent all that received injections of adrenal extracts with the exception of certain cases in which complications not connected with the treatment occurred. These will be enumerated but are excluded from the table. The material for injection was prepared, by various methods, from fresh dogs' adrenal glands obtained aseptically from animals that were being adrenalectomised, and was kept in the refrigerator. No material kept more than a week to ten days was used. As extracts of the whole gland were employed the medulla contributed something to the extracts and adrenalin was always present but in small amounts (as tested colorimetrically). On standing the small adrenalin content became still less. We have no hesitation, however, in concluding that the results were not due in any important degree to adrenalin. For 1, our experiments on rabbits and cats instituted 10 years ago showed that in animals which had developed the serious symptoms associated with adrenal insufficiency, only a transient improvement could be effected by intravenous injections of adrenalin. Other investigators have also found that life could not be prolonged materially in this way. 2, Control experiments on dogs with injection of quantities of adrenalin as great as or greater than could have been contained in the extracts yielded negative results. Three of these experiments have been included as additional controls in table 2. 3, In another series, extract injection experiments in which slaughterhouse material (sheeps' adrenals) was employed, the cortex was separated from the medulla and extracts of cortex were made. These experiments will be dealt with in a later paper. It will suffice to state here that the results were essentially similar to those in table 1. The symptoms and morbid changes in the gastro-intestinal tract appeared to be influenced still more favorably than in the series with dog's adrenal extracts, and life was prolonged in quite as large a proportion of the animals well beyond the extreme limits seen in controls; while the proportion of animals which without surpassing the maximum of the controls, that reached the higher levels of the survival period, was increased.

The dose of extract usually employed was 0.5 cc. or 1 cc. intravenously administered.

Seven dogs in table 1 out of 29 may be considered as surpassing the maximum survival period shown in the controls (tables 1 and 2, paper I), one only slightly (nearly 16 days, animal 127–6). One animal lived $17\frac{2}{3}$ days; one, $19\frac{3}{4}$ days; one, $21\frac{2}{3}$ days; one, $22\frac{2}{3}$ days; one, $27\frac{2}{3}$ days; and one, $78\frac{2}{3}$ days. No such numbers are to be seen among the controls. There is no circumstance in the injection experiments to which they can be attributed except the presence in the adrenal extracts of something which for a time can "substitute" for the specific substance produced by the adrenal (cortex). It is quite immaterial whether this substance acts by aiding in

the destruction or neutralisation of a poison or poisons produced in the absence of the adrenals, or supplies something necessary to the continued normal functioning of essential organs.

The question has already been alluded to (Rogoff and Stewart, 1927) why some of the animals and not all are favorably influenced. It cannot be stated at present that even with a potent, stable preparation which could be kept without deterioration for a time sufficient to permit a large series of animals to be tested, all of the animals would be benefited, or equally benefited. There may be individuals more susceptible than others to the beneficial effects of extracts. Some may be more capable of storing the important substance or of using it economically than others. At present it is not profitable to speculate further upon this matter.

From the way in which our experiments were performed, it is impossible that great variations should not have occurred in the effects of the extracts. In the first place, the extracts were obtained from the adrenals of different dogs. It could hardly be expected that different extracts made by the same method from identical material should necessarily be equally potent. The extracts were kept in the refrigerator and were used as long as they remained clear. About a dozen different extracts (all made by the same technique) were employed more or less in treating the series of dogs in table 1. Only a small number of animals could be treated at one time. If an extract seemed to be more successful than usual it could still be administered only to a small number of animals. Nor was there any way of knowing how rapidly a given extract, initially potent, lost potency on standing, even if it remained clear. Of the extracts mentioned in table 1, II seemed to be specially successful. At least the three animals (123–0, 123–1, and 123–3 in the table), in which it was injected, all showed long survival periods, two of them well beyond the maximum of controls. But another extract (V) was employed in two of these animals in the latter part of the experiment. Extract XIX seemed to be unusually potent, as two out of the three dogs in table 1, in which it was employed, lived much beyond the maximum seen in controls.

In the second place injurious substances may have been introduced into the blood as well as the beneficial substance, and the amount of these would, of course, vary in the different extracts and probably in the same extract when kept. Control tests on unoperated dogs were made in order to see whether any effects resulted which could be attributed to such injurious substances, but the test was negative.

Third, it has already been seen in paper IV (Rogoff and Stewart, 1928) that even with the same injection liquid (Ringer's solution) great variations occur in the effects in different dogs.

Undoubtedly, had the series been extended an additional number of long survivors could have been added to the table. But there would have been

no point in spending a large amount of time for this purpose. Our object was achieved when it was demonstrated clearly that extracts were obtainable which could be safely administered (intravenously) to dogs and which could "substitute" for the glands. We deprecate naming every active substance obtainable from an endocrine gland a "hormone". That should only be done when it is known to be given off physiologically from the gland. Very few of the active substances which have been separated from endocrine glands can at present satisfy this condition. Nevertheless, there is no harm, and a certain convenience, now that it is known that active extracts can be derived from the adrenal (cortex), in giving a name to the hypothetical body. We considered "corticin," but rejected it. It suggests, of course, a substance obtainable from cortex but does not indicate what cortex; it might be kidney or brain. The best term, in our opinion, is "interrenalin," a substance derived from the interrenal gland tissue. We have always considered "adrenin" a physiological misnomer, and the same would be true of "interrenin," although a syllable would be saved.

The proof that a substance can be extracted from the adrenal (cortex) which can prolong life after adrenalectomy has now been given. This affords an indispensable basis for all work directed to isolate and purify the substance. Till this proof was forthcoming work in this direction could not be pursued with confidence or any promise of success.

A few of the protocols will now be given in condensed form.

Dog. Male. Record number 123-0. On October 28, 1926, bodyweight 9.0 kgm.; left adrenal removed (0.70 gram). November 9, weight 9.0 kgm.; right adrenal removed at 10:30 a.m. (0.72 gram). November 10 to 12 excellent condition. On November 11 received 1 cc. of extract II, at 3:00 p.m. Large appetite. November 13, in the morning, hind legs somewhat wabbly; getting more apathetic; emesis (neutral to litmus); refused all food today. At 3:00 p.m., received 1 cc. of extract II. The heart was slowed then accelerated after the injection. At 4:15 p.m., more emesis of frothy matter (neutral); steady on legs but apathetic. November 14, at 2:00 p.m., much better; took food readily. November 15, very good condition, but yelled twice as if alarmed. At noon took a good meal (meat). At 12:30 p.m., received 0.5 cc. of extract II. In the evening violent yelling and barking fit, terminated by his eating biscuit. November 16, refused all food; more apathetic again. November 17, at noon ate a good meal (meat). In much better condition; pugnacious. Was ready for more food in the evening. November 18, condition very good; ate well. At 10:00 p.m., received 0.5 cc. of extract II. On November 19 and 20, remained in good health; appetite good; pugnacious. November 21, unchanged; received 0.5 cc. of extract II. Defecated normally. November 22, slight diarrhea (no blood). Refused food; quite apathetic. At 5:00 p.m., vomited yellow liquid (acid to litmus). At 11:30 p.m., just died; had not changed his position.

Autopsy. Pancreas considerably congested. Stomach contained bile and blood; mucosa hemorrhagic. Duodenum contained bile-stained mucus; mucosa moderately congested. Small intestine moderately congested; only a little blood in lumen.

A feature in the history of this animal, after the second adrenalectomy, was the fluctuation in the state of health and appetite. The impression was given that the animal was being picked up from time to time (by the injection?). The terminal stage was very brief. The number in the table in the column headed "began to refuse food" refers to the complete anorexia on the last day. There had been several occasions on which no food was taken for a day, but appetite was regained.

Dog. Male. Record number 123-1. This animal was treated with extract II at the same time as dog 123-0. October 28, 1926, weight 7.4 kgm.; left adrenal excised (0.70 gram). November 10, weight 7.65 kgm.; right adrenal excised at 10:40 a.m. (0.80 gram). November 11, excellent condition. Received 1 cc. of extract II. The same dose was given on November 13 at 3:00 p.m. Remained in good health on November 14. On Novemver 15, after injection of 0.5 cc. of extract II, he yelled and ran about in the cage, but ate biscuit readily. November 16, had a short "yelling" spell in the morning but ate well. November 17, ate meat readily, chewing the bones; condition excellent; normal solid stools. November 18, condition good; took food well; pugnacious; 0.5 cc. of extract II. November 19 to 21, unchanged. On November 21, received 0.5 cc. of extract II. November 22 to 24, condition remained excellent; appetite keen; injected with 0.5 cc. of extract V (a new lot). November 25 to 26, unchanged; appetite good. Weight 7.6 kgm. November 27, condition seems very good; eating well, but hind legs seemed somewhat stiff; slight muscular twitching but no weakness. Received 0.5 cc. of extract V. November 28, condition excellent; marked pugnacity. November 29, less energetic; but ate a good meal. At 4:00 p.m., received 0.5 cc. of extract V. November 30, lies about more than usual; more apathetic; but eating well. Can run well, although somewhat stiff at first. December 1, seemed more lively than yesterday. Ate a fair meal (meat) at noon. At 1:00 p.m., injected with 0.5 cc. of extract V. He walked well. December 2, died early this morning. The autopsy showed moderate congestion of portions of gastro-intestinal tract but no blood in the lumen. Pancreas considerably congested.

The final stage was even shorter than in dog 123-0; in fact it cannot be said that there was any definite stage characterized by anorexia. In dog 123-3 also, this stage was very brief. In a number of the injection experiments we received the impression that the animals artificially kept alive beyond the maximum survival period of the controls, were liable to collapse more suddenly than the latter. Possibly an increase in the number of injections at this point might have caused renewed benefit, but this was not done as it seemed preferable first to finish the series according to the prearranged plan.

Dog. Male. Record number 123-3. October 29, 1926, weight 10.0 kgm.; right adrenal removed (0.70 gram). November 9, weight 9.65 kgm. November 11, weight 9.65 kgm.; at 10:00 a.m., left adrenal removed (0.80 gram). November 12, very good condition; took food, but soon vomited. Received 1 cc. of extract II. November 13, good condition. November 14, took food but some emesis (bilious). November 15 and 16, excellent condition; eating well. On November 15, 0.5 cc. of extract II. November 17 to 21, unchanged. On November 21, received 0.5 cc. of extract II.

November 22 to 25, unchanged; good appetite. On November 24, received 0.5 cc. of extract V (new lot). November 26, weight 9.2 kgm. Good condition, but wabbles a little at first in walking. Less energetic. Fair appetite; some emesis. Received 0.5 cc. of extract V. November 27, quite shaky and more apathetic; took fair meal at noon. At 6:00 p.m., weak. November 28, better than yesterday, but refused all food; hind legs weak. Received 0.5 cc. of extract V. November 29, died during the night.

Dog. Female. Record number 125-7. December 8, 1926, right adrenal excised; weight 6.95 kgm. The weight was taken many times up to February 13, 1927 and only varied slightly from 7 kgm. February 15, left adrenal excised at 10:00 a.m.; weight 7.2 kgm. February 16, condition very good; ate well. Injected with 0.5 cc. of extract XVI. This was used throughout in the same dose. Weight 6.7 kgm. February 17, unchanged. February 18, had yelling and racing fits all day but ate well. Injection. February 19, some yelling, but ameliorated. Ate well. February 20, seems well, but did not eat much of meal (bread and milk). Injection. From February 21 to March 3, remained in excellent health, eating (bread and milk, meat) well. Injected on alternate days. Weights 7.25, 7.2 kgm. March 4, appetite poor, but finished the meal slowly. Injected. March 5, good condition, but ate more slowly. March 6, unchanged. Still eats (meat, especially), but appetite is not so good. Injected. March 7, weight 6.9 kgm. Fairly active. Emesis (bilious) in forenoon, but ate noon meal (meat). Up to March 13, no change. Continues to eat meat well but since March 11 has not cared for biscuit which she liked previously. Injections as usual, the last being on afternoon of March 12. March 14, wabbles in attempting to walk; apathetic; appetite small but was coaxed into eating some ham (not retained). Prefers not to get up, but no acute symptoms. Weight 6.55 kgm. March 15, dead in the morning. There was practically no congestion of gastro-intestinal tract. There was no evidence during life that the animal was either in heat or pregnant and this was confirmed at the autopsy.

Dog. Male. Record number 127–9. February 17, 1927, left adrenal removed; weight 10.2 kgm. February 21, weight 10.5 kgm. February 28, weight 10.1 kgm. March 8, right adrenal removed; weight 10.2 kgm. The animal remained in excellent condition till March 18. On alternate days received 0.5 cc. of a fresh extract (XIX), beginning March 9. On March 19, seems somewhat less lively; slightly unsteady on hind legs. Ate biscuit readily; in the evening seemed normal. Up to March 24, no change. Hair coming out; does not care for biscuit any more but takes meat. March 25, not quite so active as heretofore, but eats (meat), although with diminished appetite. Injections continued as before. March 26, eats meat, but appetite lessened; more apathetic; does not wish to leave the cage. March 27, refuses all food; passing bloody mucus from anus; very apathetic. Walks well when he does get up. At 3:00 p.m., received the last injection. On the morning of March 28, he was dead.

Dog. Male. Record number 128–0. February 18, 1927, left adrenal removed; weight 11.8 kgm. Up till March 7, the weight remained about 11 kgm. March 8, right adrenal removed at 10:30 a.m.; weight 11.3 kgm. Till March 15, the condition of the animal was very good; appetite excellent. Received injection (0.5 cc.) of extract XIX on alternate days throughout the whole period of survival. Seems a bit "off" but ate well. Somewhat unsteady on hind legs. March 17 to 19, very good condition; eating well. Injection continued on alternate days. March 20, less alert. Refused biscuit, which he always took before. Ate meal of bread and milk, but later vomited it (with bile). March 21, apathetic. Refused food till coaxed. Weight

11.35 kgm. Unwilling to get up, but walks all right. March 22 to 24, unchanged. Eats meat but not with great appetite. Refused bread and milk. Less vigorous than a few days ago. March 25 to 28, more alert; eats meat with good appetite. Is a little stiff in walking. A little blood in stools. March 30, lacks strength in hind legs; wabbles when walking. No food taken today. More apathetic. Some emesis (bile). At 3:00 p.m. and 9:00 p.m. passed some blood from bowel. March 31, at 2:00 a.m. is losing consciousness. Died before 6:00 a.m.

Dog. Male. Record number 124–1. November 5, 1926, weight 10.55 kgm.; right adrenal excised. November 9, weight 10.3 kgm. November 26, weight 10.4 kgm.; left adrenal excised at 11:00 a.m. November 27, good condition; ate well. Received 0.5 cc. of extract V. November 28 to December 4, very good condition; eating well. Received on alternate days 0.5 cc. of extract V. December 5, ate only a little (bread and milk), otherwise unchanged. Received 0.5 cc. of extract V. December 6, some shivering, which passed off. Ate well. December 7 and 8, excellent condition. Received 0.5 cc. of extract V on each of these days. December 9 to 15, condition remained excellent; appetite very good. On alternate days received 0.5 cc. of extract V. On December 9, weight 10.5 kgm. On December 16 finished extract V; henceforth he got extract XII on alternate days (0.5 cc. to 0.75 cc.) till December 23, when the injections were discontinued. Weight December 23, 10.85 kgm. His condition remained good. December 23, 1926 to January 2, 1927, no change seen. He begs as usual standing on hind legs, and eats ravenously. On December 31, weight 10.55 kgm. On January 2, less active; some apathy; and hind legs weaker but ate fairly well. January 3, distinctly more apathetic and unsteady in walking; eats meat, but with less appetite than usual and neglects the bones. Lies about more than usual. Some emesis (bile) this forenoon. At 1:30 p.m., injection (1.25 cc. of extract XII). At 3:00 p.m., ate meat and seemed improved. At 4:30 p.m., decidedly improved, walking on hind legs; not at all wabbling and much more alert. The injection seems to have picked him up completely. On January 4, he was normal and eating well; continued in excellent condition till February 6. Normal behavior towards female dogs. Weights on January 10, 10.2 kgm.; on January 17, 10.1 kgm.; on January 24, 10.4 kgm.; on January 31, 10.15 kgm.

From February 6 onwards, he became quieter, his appetite became less keen although he continued to eat meat. On February 7, weight 9.85 kgm. On February 10, not much change. On February 11, he was decidedly below par. Apathetic, but not weak. February 12, refused meat at noon, but was coaxed to eat a few of the best morsels. Apathy increased. A little matter in the corner of the eyes (often seen in last stages); head hanging down; skin of cheeks and jaws sags as is often seen when asthenia is developing. At 5:30 p.m., he seemed more alert; walked about more and was not in the least wabbly. February 13, found dead at 9:30 a.m. He must have died early in the morning. The most striking feature in the post-mortem appearances was the complete absence of congestion in the gastro-intestinal tract or of blood in the lumen, as indicated in the table. Another feature was the great size of the parathyroids. We simply note this without attempting to explain it.

One or two points in the protocol require some discussion. When injection was intermitted the animal continued in good health for about 10 days. Was he then really being kept alive by the injections? The only answer, but in our opinion a sufficient one, is that there is nothing else to which the long survival can be attributed. At the operations the adrenals were seen

to be completely removed. The completeness of the removal was carefully verified at autopsy. No adrenal accessory was discovered by most careful search. This is true of all the dogs both the control animals and the animals treated by injections of Ringer's solution, of adrenal extracts and in other ways. To suppose that in those 29 dogs of table 1 we accidentally hit upon 6 or 7 which lived distinctly longer (in some cases very much longer) than the maximum seen in nearly 150 controls is not reasonable. That an animal being kept alive by injection of extracts should live 10 days after intermission of the treatment before showing characteristic signs of deterioration is not at all improbable. For that is about the time when many dogs begin to develop the fatal symptoms after adrenalectomy. If the capacity of many dogs to live in good health as long as that after removal of the adrenals is due to active substance stored elsewhere than in the adrenals (possibly but not necessarily in the genetically similar tissues of the sex glands), it may be that the active substance in the extracts is also stored. If there is not an actual storage of the active substance which enables the animal to go on living for a time after injections have been discontinued, there may be a more or less durable change in certain functional activities due to the extracts and outlasting their administration. Or, if the survival of controls for a time is due to the slowness with which the intoxication increases to the point at which definite symptoms appear, then the same thing might be expected to happen on intermission of injection of extracts. The prompt recovery of the animal from an obviously serious condition when the administration of extract was resumed corroborates the conclusion that his survival is to be attributed to the extract. That he should live more than a month after the last injection could not have been anticipated. We see no reason to doubt that this was due also to the previous injections. He was not being kept alive by adrenal tissue for none was found, as already mentioned, and he died at last and with little, if any, warning, with symptoms characteristic of adrenal insufficiency. He would be as much out of place among the controls, with a survival period of 30 to 40 days, as with the full period of nearly 80 days. The only new factor is the adrenal extract. There the matter must be left at present. The sudden end is worthy of note, as is the fact that some food was taken almost up to death. The complete absence of the common pathological changes in the gastro-intestinal tract is a feature which has been already mentioned.

Six animals which received injection of extracts are not included in the table because of certain complications. Three were pregnant, and lived 17 days, 4 hours; 24½ days and 33½ days respectively. One animal had been used before the first adrenalectomy for testing any possible harmful effects of extracts. It was also in quarantine on account of mange and respiratory trouble. This animal lived 7 days, 19½ hours. One, very

mangy before the first adrenalectomy and remained so after the second operation, lived 5 days, 15 hours. In another dog, which lived 5 days, 4½ hours, the right adrenal was very deep seated under the liver; the cava was torn, necessitating its repair, and involving much more trauma than usual.

As illustrating the immediate beneficial effect of transfusion of adrenal vein blood from one normal dog into a second (adrenalectomised) dog, a protocol may be given. We do not think that blood from the adrenals is different from blood from any other source in this respect and we have seen ordinary defibrinated blood produce a similar effect. It is quite unknown whether the substance which has life-prolonging power is given off to the blood. The epinephrin always contained in blood from the adrenals might or might not have a temporary beneficial influence, even in the small concentration normally present. But all the evidence goes to show that adrenalin injections do not materially prolong life. Many of the experiments of Tournade on anastomosis of the adrenal vein of one animal with the jugular of another show nothing more than has been known for a considerable time, that epinephrin is given off from the adrenals.

Dog. Female. Record number 85–9. September 15, 1924, right adrenal excised. September 22, left adrenal excised. Took food up to September 25, when she refused it; was weak though able to walk. Temperature 36.9°C. September 26, very asthenic. Temperature 33.4°C. At 3:30 to 4:00 p.m., made a cava pocket in a large (normal) dog and united the pocket by cannula with the jugular vein of dog 85–9. Transfused 10 to 15 minutes. Then collected blood from the pocket and after defibrination injected it into dog 85–9 (100 cc.) at 4:30 to 5:00 p.m. At 5:05 p.m., the temperature was 37.8°C; the dog defecated (watery stool) and drank water. Able to stand better. At 9:00 p.m., temperature 34°C. A mixture of Ringer's solution and adrenal blood (100 to 125 cc.) was injected. Respiration and pulse rate increased. Continued to urinate freely. From 10:00 to 10:40 p.m., she began to walk about. Temperature 37.1°C. At 10:50 p.m., injected 800 cc. Ringer's solution with dextrose to make up 2.5 per cent. Free urination. At 11:20 p.m., temperature 37.8°C. At 11:35 p.m., took a run in corridor. No weakness in legs. September 27, died early this morning.

As soon as it appeared that positive results were being obtained, with injection of extracts of dogs' adrenals, that is, survival periods lengthened beyond the maximum seen in the controls, it was decided to accumulate another series of controls (untreated adrenalectomised animals, either male, or females, non-pregnant and not in heat). Only in this way could the possibility be excluded that increased practice in the operation with improvements in the technique might be responsible for longer survival, which we might be attributing erroneously to the extracts. Table 2 displays the results of 36 new controls. It will be seen that they are essentially the same as those in tables 1 and 2 (paper I). The dogs were males except numbers 121-1, 121-3 to 121-6 (inclusive), 122-2, 122-5, 123-2, 123-4, 125-8 to 126-0 (inclusive), and 128-2. Included in the table are 8 dogs (121-3 to

121-6, inclusive, 122-2, 122-5, 123-2 and 123-4), which were injected with a commercial preparation of corpus luteum. It will be seen from the condensed protocol of one of these dogs (122-2), which survived 12 days, 6½ hours, that the results were negative, no noticeable effects of any kind being produced. The experiments were, therefore, considered as additional controls.

Dog. Female. Record number 122-2. October 6, 1926, weight 9.3 kgm.; right adrenal excised. October 27, weight 9.2 kgm.; left adrenal excised at 10:00 a.m. October 28, good condition; 1 cc. commercial corpus luteum extract injected intravenously. October 29, had two yelling spells; injected 1cc. corpus luteum extract. October 30, excellent condition; ate well though she had a yelling spell during the meal; injected 1 cc. corpus luteum extract. From October 31 to November 6, she continued in very good condition, eating well and behaving normally except that on two or three occasions she had hallucinations; 1 cc. of corpus luteum extract was administered, intravenously, daily. November 7, emesis (bile); active but not keen for food (bread and milk; dog biscuit); in the evening she appeared somewhat apathetic and stretched a good deal. November 8, total anorexia; emesis (bilious; alkaline to litmus); asthenic; at 2:00 p.m., very asthenic; diarrhea (bloody stool); 3:00 p.m., comatose; died at 4:30 p.m. *Autopsy.* Liver, spleen and kidneys congested, pancreas very markedly congested. Uterus small (virgin); ovaries small and no visible corpora lutea. Stomach contained very bloody liquid; the mucosa was hemorrhagic and three small ulcers, extending through to peritoneum were present in the pyloric end. In the small intestine the contents were bloody throughout, increasingly so downwards; the entire mucous membrane was exceedingly hemorrhagic. The large intestine and rectum were empty; mucosa moderately congested.

Three animals (129-0, 131-4 and 131-6) received intravenous injections of adrenalin in amounts greater than could have been present in the adrenal extracts. The results were also considered negative and the animals included among the controls. One male dog (122-4) belonging to a group intended for injections was observed to be particularly vigorous and gave the impression that he might live a long time. He was, therefore, allowed to live without treatment, as a control. The survival period was the longest we have yet seen in untreated dogs, namely, 16 days and 6 hours. The animal is not included in the table because it seemed to us that if that were done the rest of the group to which he belonged ought to go in also, which was impossible. The matter is of no consequence, but it is clearly demonstrated that a dog receiving no treatment can survive the removal of the second adrenal as long as 16¼ days. This is the maximum period seen in our controls, (at least 120).

SUMMARY

Proof is given that extracts of adrenal cortex can prolong the period of survival of dogs after adrenalectomy, beyond the maximum seen in control, untreated animals.

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THE ENERGY METABOLISM OF WOMEN WHILE ASCENDING OR DESCENDING STAIRS

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Walking, chiefly on a level but likewise in no inconsiderable part up and down stairs, forms a feature of every individual's daily activities, in spite of the modern developments in transportation by automobiles and elevators. As the result of a number of satisfactory researches on level walking it is possible to compute the energy involved in such exercise, and the total energy output of the sedentary individual can be computed if one knows the basal metabolism, the hours of sleep, the hours of sitting and the hours of walking on the level. Since even the sedentary person must to some extent ascend and descend stairs during the day, it is desirable to be able to add to the energy output thus computed that involved in going up and down stairs. Knowledge of the energy requirements for these types of muscular activity, aside from having a physiological importance, seemingly has a great practical value. Thus, this knowledge is of assistance to the physician in prescribing definite amounts of exercise, such as either the considerable amounts necessary in reduction cures or the more subtly graduated work advisable in heart cases. The chief object of our study was therefore to determine for the use of the physician and the physiologist some simple factor which, when multiplied by the body weight of the individual and the vertical distance travelled, will give a direct measure of the total metabolism involved in the ascent or descent of stairs. A large number of women volunteered as subjects for this research and our observations were accordingly made only on women.

Previous investigations. The literature on the energy requirements of walking has been extensively reviewed up to 1922 (Benedict and Murschhauser, 1915; Smith, 1922), and we shall here discuss only the publications appearing since that time. Aside from the observations on Frau Durig (Durig, 1906) and those on women reported by Bedale in 1924, by Cathcart and his colleagues in 1927, and by Smith and Doolittle in 1925, all the measurements dealing with horizontal and grade walking have been made upon men. Magne (1920), (1922) studied the metabolism

of a man walking up a smooth, inclined path with elevations only to 15 degrees, i.e., but about half the inclination of the ordinary stairs. Both the work of ascent and descent were studied. Langlois (1921) described a treadmill or rolling platform which was used by Chailley-Bert (1921) and two years later by Faillie (1923), (1924). Faillie studied the work of descent at various angles, but since in his experiments the walking was performed upon a smooth surface, his results are not directly comparable with observations obtained in staircase experiments.

Employing the Douglas bag method, A. V. Hill and his colleagues (Campbell, 1924; Furusawa et al., 1924, 1925, 1927; Hill et al., 1922–1927; Long, 1925; Lythgoe and Pereira, 1925; Sargent, 1926) carried out a series of experiments between 1922 and 1927 in which they studied particularly the oxygen consumption during running. Marching on a mountain was reported by Rabbeno (1923), (1925) and the marching of soldiers was studied by Cathcart (1923) and his colleagues. Loewy and Schroetter in 1925 published their results on horizontal walking and the ascent of mountains, and in 1926 articles by Fleisch (1926a), (1926b), Studer and Sigrist appeared from the laboratory of Hess, describing the Fleisch treadmill and experiments with it. Finally, in 1927, there have appeared articles by Atzler, and by Atzler and Herbst dealing with treadmill walking and the pulling and pushing of loads.

We are particularly interested, however, in the special phase of walking represented by staircase climbing, for undoubtedly the muscular effort expended in the ascent of stairs is altogether different from that expended in walking on an inclined plane or even on the uncertain terrain of a mountain path. The earliest report that we have found in which attention has been given to staircase climbing is that of Coulomb (1797). He, however, did not study the respiratory exchange, and the first attack on the staircase problems dealing with respiratory exchange is undoubtedly that of Waller (1918, 1919a, 1919b) and De Decker (1919), who measured the carbon-dioxide production. Smith (1922), who has summarized Waller's findings, points out that the values for the mechanical efficiency show wide variations, ranging in 17 experiments with A. D. W. from 24.8 to 41.6 per cent (Waller and De Decker, 1919), but averaging 33 per cent with 12 subjects (Waller, 1919a).

In 1922 Peabody and Sturgis devised a stair-climbing treadmill with which they studied the oxygen consumption of men. In 1923 Lupton published the results of a series of experiments in which the oxygen consumption during the ascent of 78 steps was determined on a number of men, and in 1924 Collett and Liljestrand reported experiments with the staircase treadmill of Stenström in which the oxygen consumption of one woman and one man were studied.

Plan of research.[1] A study of the metabolism of women during the ascent and descent of stairs has a physiological interest in that the altogether different gaits of men and women suggest a possible difference in their mechanical efficiency in this common daily exercise. Smith's evidence (Smith and Doolittle, 1925) is against a sex difference, so far as horizontal walking is concerned, but the question with reference to stair-climbing is still to be settled. Our research was therefore primarily devoted to a study of the energy expenditure of women during stair walking, chiefly during ascent but likewise during the descent of stairs. Incidental thereto certain experiments were made while the women were standing or walking on a level. These data have been used to compute the work of ascent over and above that involved in standing and in forward progression.

For stair-climbing ideally an escalator is desirable. Such not being available, we made use of the new Cornelia Clapp Laboratory at Mount Holyoke College which, including the tower, has altogether six flights of stairs. A mountain railway near the college, with a long, unbroken flight of steps (522 steps), was also available but could not be used during the winter weather. The experiments were therefore first made at the Clapp Laboratory.

Apparatus used. Heretofore practically all experiments on walking have been carried out by an elaborate technique, which of itself restricted the collection of data. With the development of new apparatus at the Nutrition Laboratory a much simpler technique was possible. Indeed, it was found that by having the subject breathe into an apparatus[2] light enough in weight to be carried in the hand or as a "knapsack" on the back, walking on a level at least could be performed without difficulty. This knapsack type of apparatus was subsequently modified so that there would be an increased absorption area of soda-lime to remove the carbon dioxide produced by the subject, and so that the operator would carry the greater part of the weight of the apparatus, the subject supporting only the weight of a portion of the tubes leading to the reagent can, a noseclip, and a mouthpiece. A view of the apparatus as finally developed for use in the walking experiments at Mount Holyoke College is shown in figure 1.

The metal can, *A*, is divided into two compartments by a partition (not seen in fig. 1), contains approximately 1400 grams of soda-lime, and is covered at the top with a light-weight rubber bathing cap, *C*. Air enters

[1] Acknowledgment is due Miss Mary D. Finn for her assistance in the early experiments and Prof. A. H. Turner for her helpful coöperation throughout the investigation.

[2] This apparatus was publicly demonstrated at a meeting of the Harvard Medical Society at the Peter Bent Brigham Hospital in Boston, Massachusetts, on February 24, 1925, and at the Carnegie Institution of Washington, Washington, D. C., in December, 1925.

a pipe at the bottom of the can on one side, passes up through the soda-lime in this compartment, over the top of the partition, down through the soda-lime in the other compartment, and out on the other side through a hole near the bottom of the can, thus travelling the maximum distance

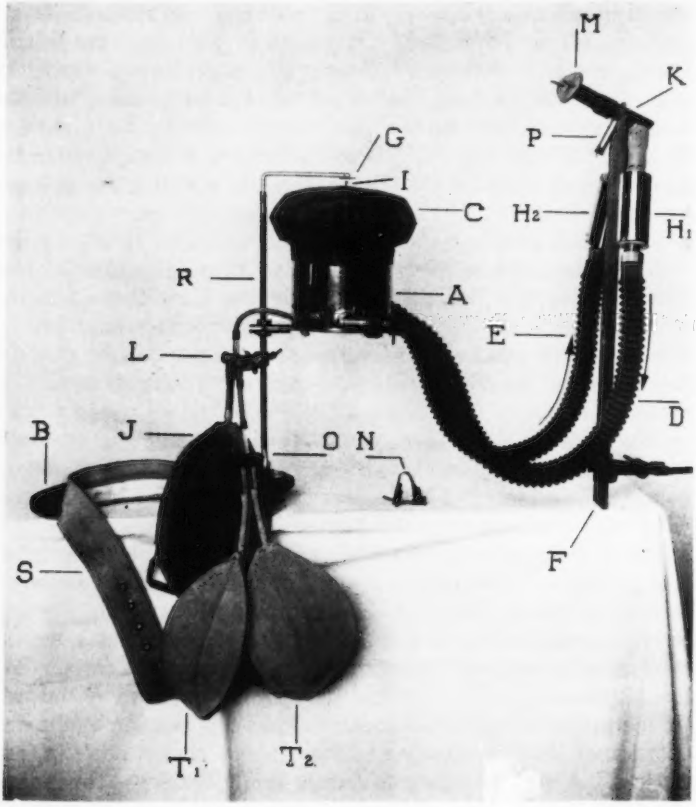

Fig. 1. A respiration apparatus for determining the energy metabolism of walking. The soda-lime container, A, and the oxygen bags, J, T_1 and T_2, are carried by the operator. The subject breathes through the mouthpiece, M, and the accompanying tubes, the weight of which is supported by the thin wooden strip, F, held in the subject's hand. For details see text description.

through the soda-lime and enabling the complete removal of carbon dioxide. The soda-lime container, A, is attached to a rod, R, supported on a light-weight wooden board, B, shaped somewhat like a half-moon and strapped around the operator's waist by means of the strap, S. Additional straps

(not shown in fig. 1) going over the operator's shoulder from the upright rod, *R*, hold the apparatus steady. Two corrugated rubber tubes, *D* and *E*, each approximately 140 cm. long,[3] lead from the reagent can, *A*, under the arms of the subject to the mouth. At the end of these tubes are two light-weight metallic valve housings, H_1 and H_2, which are attached to a light metallic piece, *K*, connecting with the rubber mouthpiece, *M*. The short tube, *P*, projecting at right angles from the metal piece, *K*, and fitted with a rubber stopper at the end, serves as a trap for saliva. A thin wooden strip, *F*, 60 cm. long, notched at one end to fit under the metallic piece, *K*, is held in the hands of the subject to relieve the lips of any undue pull from the weight of the tubes. A noseclip, *N*, closes the nose. The supply of oxygen is held in three rubber bags (common basket-ball bladders). In one of these bags, *J*, is placed an unmetered amount of pure oxygen, approximately 3.5 liters. In each of the other two, T_1 and T_2, is placed a metered volume of oxygen. A stop-watch completes the equipment. The operator carries the greater part of the weight of this apparatus.

At the beginning of the experiment the subject breathes oxygen-rich air, and the contents of the first unmetered bag, *J*, are used during the period of preliminary adjustment. When the index button, *I*, on top of the cap, *C*, just fails to touch the index plate, *G*, at the end of a normal expiration, the time is noted and the stopcock *L* is turned to connect the apparatus with the first metered bag, T_1. The stopcock, *O*, has previously been turned to allow connection only with bag T_1. The time required for the complete consumption of the oxygen in the bag T_1 is noted on a stop-watch and the stopcock, *O*, is then turned to the second metered bag, T_2, the time being again noted when the contents of this bag are completely used. Under these conditions two experimental periods, immediately succeeding each other and presumably exact duplicates, are carried out which serve as admirable checks on each other.

Metering the oxygen. Aside from the time involved, the only factor actually measured in experiments of this type is the amount of oxygen consumed. For this measurement nothing has been found better than a simple plunger pump with accurately fitting piston and known length of stroke (Benedict and Benedict, 1923a, 1923b; Benedict, 1924, 1925b, 1927a, 1927b, 1928). The Collins form of pump[4] has a constant length of stroke of 200 mm. and an apparent volume of 369.5 cc. In our walking experiments oxygen was drawn into the pump over calcium chloride or directly from a cylinder of highly compressed and therefore dry gas, but it is somewhat simpler to deal with saturated air. A definite number (usually 6 to

[3] In the figure but one-half this length is shown.

[4] This pump, as indeed the entire apparatus, may be secured from W. E. Collins, 555 Huntington Avenue, Boston, Mass.

10) pumpfuls of oxygen were introduced into each of the rubber basket-ball bladders (T_1 and T_2) which had previously been deflated to the normal flat position. These bags were connected with a 2-way stopcock, O, and the contents of either bag could be discharged into the respiration apparatus by proper turning of the stopcock and by applying gentle pressure to the bag. Since with this technique the volume of oxygen in these bags is metered *prior* to the beginning of the experiment and the temperature of the pump barrel as well as the barometric pressure are recorded, the volume of each bag may, if desired, be reduced to 0°C. and 760 mm. and converted to calories (by assuming that each liter of oxygen at 0°C. and 760 mm. and at a respiratory quotient of 0.82 represents 4.825 calories) *before* the experiment begins. This reduced volume or its equivalent in calories, compared with the time required for its consumption, is a direct measure of the metabolism. Usually the ordinary basket-ball bladder will hold 10 pumpfuls of oxygen without significant back pressure due to the distention of the rubber, but the internal pressure should under no circumstances be more than the equivalent of 1 cm. of water. In walking experiments, when the respirations have a somewhat greater amplitude than in rest experiments and there is a tendency to irregularity in breathing, the moment when the oxygen from the metered bag is completely absorbed is not so sharply defined as in lying experiments, but the technique is essentially that for basal metabolism experiments (Benedict and Benedict, 1923a, 1923b; Benedict, 1924, 1925a, 1927b, 1928).

Subjects. All the subjects were women students at Mount Holyoke College and all, with one exception, were previously trained on this particular form of apparatus. They were studied only in the non-menstrual period, usually in the post-absorptive condition, and wore the ordinary school clothes. The ages, weights and heights of the subjects are given in table 1.

Horizontal walking experiments. The recent paper of Smith and Doolittle (1925) on the energy expenditure of women during horizontal walking leaves little to be desired. Our data on horizontal walking, which were secured simply as incidental to the stair-climbing research, are, however, here recorded. All of our horizontal walking experiments were made indoors in the 45-meter corridor of the laboratory. The distances walked were measured in two ways. From the rate of walking (which was always known exactly by the use of a metronome) and the duration of the experiment the number of steps were computed and then multiplied by the average length of step. A second method employed a small, rubber-tired wheel, 29.7 cm. in diameter, connected with a revolution counter. An assistant brought this wheel in contact with the floor at the spot where the experiment began, dragged or pushed it along as the experiment continued, and lifted it from the floor at the spot where the experiment stopped.

The points where the experiment began and ended were marked by dropping small bags of shot.

After a number of preliminary experiments the following routine was decided upon for the horizontal walking experiments. The apparatus was filled with oxygen-rich air and the oxygen bags were attached. Since Smith (1922) has shown that the period of transition from standing to walking causes many changes in the metabolism, we attempted to eliminate the effect of the transitional period by having the subject, prior to the actual measurement of the metabolism, attach the noseclip, breathe through the mouthpiece, and walk in the corridor for from 3 to 4 minutes. Oxygen was of course used out of the apparatus during this preliminary walking, and the bag of unmetered oxygen (J, fig. 1) was employed to make the

TABLE 1

Age, weight and height of subjects

SUBJECT	AGE	WEIGHT (CLOTHED)*	HEIGHT
	years	*kgm.*	*cm.*
I	21	70.5	164
II	19	63.4	164
III	20	61.2	165
IV	21	59.8	172
V	25	54.9	179
VI	22	47.3	153
VII	23	48.8	158
VIII	20	56.8	154
IX	20	56.7	166
X	19	50.5	156
XI	18	65.6	162
XII	18	50.2	158

* Average weight of clothes, 1.5 kgm.

initial adjustments in the degree of distention of the rubber bathing cap. The experiment proper then began. The stop-watch was started, a bag of shot dropped, and the stopcock, L, turned from the unmetered to the metered bag, T_1, all unknown to the subject. When the oxygen in the first metered bag was used and the bathing-cap had just failed to come in contact with the index plate at the end of an expiration, the time was again noted and the stopcock turned to the second metered bag, T_2. The experiment then continued until the oxygen in this second bag was used, when the time was again recorded and the subject stopped walking.

Total energy expenditure per horizontal kilogrammeter. The results of the horizontal walking experiments with our different subjects are given in table 2, expressed as total calories per horizontal meter per kilogram of body weight (using the weight as walked). The total energy cost in

walking a given distance will obviously be made up of two factors, the actual cost of living or the standing metabolism during this period and the energy involved in the effort of transporting the body a given distance. When the rate of walking a certain distance is slow, the first factor plays a much larger rôle than when the rate of walking the same distance is fast, because of the difference in time required. For this reason strict comparison cannot be made of the values given in table 2, for the time required to walk one meter (or the cost of living) is greater at the rate of 34 meters than at 65 meters per minute, and the values given in table 2 for the heat production per horizontal kilogrammeter therefore give only a general picture of the effect of the different speeds. Indeed, the values are to be considered only as approximate, to be used for multiplying the body

TABLE 2

Total heat production per horizontal kilogrammeter

SUBJECT*	WEIGHT AS WALKED	GRAM CALORIES WHEN WALKING AT		
		34 meters per minute	65 meters per minute	89 meters per minute
	kgm.			
I	70	1.13	0.79	
II	65	1.35	0.74	0.83
III	62	1.17	0.80	
IV	61	1.20	0.83	0.92
V	55	1.11	0.78	
VI	49	1.10	0.81	
Average.....	60	1.18	0.79	0.87

* All subjects were post-absorptive.

weight by the distance in order to compute the total energy expended in, for instance, a constitutional.

The seemingly high values at the low rate of 34 meters per minute are undoubtedly due in some part to the rôle played by the standing metabolism during the time of walking. When the subjects walked at twice this speed, the heat production per horizontal kilogrammeter was considerably less, on the average 0.79 as against 1.18 gram calories. At the high speed of 89 meters per minute inefficiency is clearly proven, for the cost per kilogrammeter is higher than at 65 meters. The average rate of walking of an ordinary person, however, would be not far from 65 meters per minute, and in general the total expenditure of the average woman walking at this rate (about two to two and one-half miles per hour) can be estimated by multiplying the distance walked in meters by the body weight in kilograms by the factor 0.79. Were we to take into consideration, as

we should, the excellent experiments of Smith and Doolittle (1925) and average with our data their results obtained at the 60-meter rate, the factor would be more properly 0.77 gram calorie per horizontal kilogrammeter. This factor we recommend. For those who prefer to use the English system, the body weight in pounds may be multiplied by the distance in miles and by 0.56 large calorie, which is the average factor obtained from our own and Smith's series.

Increment in energy expenditure per horizontal kilogrammeter. A factor of much use in physiology, particularly in the physiology of walking, is the actual increment in metabolism above the standing metabolism caused

TABLE 3

Increment in heat expended during horizontal walking over that during standing

(Values in gram calories per horizontal kilogrammeter)

SUBJECT*	METERS PER MINUTE		
	34	65†	89
I	0.65	0.53	
II	0.72	0.49	0.63
III	0.61	0.50	
IV	0.68	0.59	0.71
V	0.58	0.49	
VI	0.62	0.54	
Average	0.64	0.52	0.67
Smith's values‡	0.53	0.49	0.55

* All subjects were post-absorptive.

† Another subject (VII), not post-absorptive, walking at 42 meters per minute, showed an increment of 0.53 gram calorie.

‡ Smith and Doolittle, Journ. Biol. Chem., 1925, lxv, 672. Smith's values are for speeds of 30, 60 and 90 meters per minute.

by walking or transporting a known weight over a definite distance on the level. This factor has been computed in nearly all researches on walking. The total energy expended in walking, as measured by the oxygen consumption, is first obtained. From this is deducted the energy expended in standing (determined prior to the work), and the remainder is divided by the weight (in kilograms) transported times the distance in meters, which gives the calories per horizontal kilogrammeter. The results thus obtained with our subjects are reported in table 3 for the three different speeds, and Smith's average values are likewise given for comparison.

The increment is distinctly higher at the rates of 34 and 89 meters per minute than at 65 meters, thus bearing out the previous inference that the

optimum walking is done at about 60 meters per minute and that sauntering is uneconomical, so far as the transporting of body material over a given distance is concerned. Our values differ noticeably from those of Smith at the low and high rates of walking. Nine subjects were studied in Smith's series and only six in ours. The variability among the values for Smith's subjects is greater than with our subjects, however, and we believe that the somewhat closer agreement between our subjects indicates that our technique was adequately satisfactory. The lack of agreement between our average values and those of Smith at the low and high rates of speed is difficult to explain other than that both speeds are uneconomical or call for more extraneous compensating movements by the individual. At the most normal rate of walking (60 to 65 meters) the two series agree remarkably well, and an average value of 0.50 or 0.51 might easily be accepted. This average is close to that of 0.55 found with men (Benedict and Murschhauser, 1915), although it is perhaps surprising that women, whose gait is entirely different from that of men, should have so nearly the same factor representing the increment in energy required to move the body in a horizontal direction.

Respiration rate as affected by walking. During these horizontal walking experiments a study was likewise made of the respiration rate. It was found that the rate changed from about 15 respirations per minute while the subject was standing to about 24 or 25 respirations during walking, with only inappreciable differences caused by the different rates of walking.

Staircase climbing experiments in the Cornelia Clapp Laboratory. The stairway in this laboratory includes six flights with four landings, is roomy and well ventilated, and has an average temperature of 20°C. The angle of the steps on the first four flights is 31.7° and in the tower or the last two flights, 34.5°.

The rate of ascent was purposely adjusted to about 72 and 92 steps per minute, representing a vertical ascent at the rate of about 10 and 13 meters per minute, respectively. A metronome (usually carried by the assistant, rarely by the subject herself) was used to indicate the rate of climbing. From the number of steps climbed and the measured height of the steps the actual vertical distance ascended was computed.

Even with the relatively high staircase available in Clapp Laboratory, however, the entire ascent was made on the average in a little less than two minutes. Since from the work of Smith (1922) it is clear that at least one-half minute, if not considerably more, is required for the metabolic adjustment of the body to walking after standing, this period of adjustment was wholly included in the 2-minute period of measurement and the transition thus played a maximum rôle in these staircase experiments. To avoid this period of transition in walking experiments on the ordinary flight of stairs in any building other than an American sky-scraper is out of the question.

The ideal conditions would be to have the subject climb a long flight of stairs or an escalator and not begin the measurements until the metabolism had reached the climbing level. This was possible only later in the season, when access to a mountain railway could be had.

TABLE 4

Total metabolism and respiration rate during the ascent of stairs (Clapp Laboratory)

SUBJECT	WEIGHT AS CLIMBED	NUMBER OF EXPERIMENTAL PERIODS	RESPIRATIONS PER MINUTE		VERTICAL METERS CLIMBED PER MINUTE	TOTAL O_2 CONSUMED PER MINUTE	GRAM CALORIES PER VERTICAL KILOGRAM-METER OF ASCENT
			Standing	Climbing			
Standing start, 72 steps per minute							
	kgm.					*cc.*	
I	72	16	21.2	25.5	10.5	1,150	7.4
II	64	10	14.3	21.3	9.1	952	7.9
III	63	11	8.6	14.0	10.5	1,100	8.1
VIII	60	19	5.6	7.7	9.7	1,290	10.8
IX	58	19	14.0	25.0	9.1	934	8.6
X	51	17	16.3	25.7	9.6	942	9.3
VII	51	8	13.7	24.0	9.0	806	8.5
VI	49	11	20.0	33.0	8.6	841	9.6
Average.....	58	111	14.2	22.1	9.5	1,002	8.8
Walking start, 72 steps per minute							
I	72	7	21.2	27.3	9.7	1,302	9.1
III	63	5	8.6	14.5	9.9	1,342	10.4
VIII	60	4	5.6	9.8	10.0	1,371	10.9
IX	58	3	14.0	29.2	8.1	1,114	11.4
VI	49	4	20.0	33.3	7.5	889	11.6
Average.....	60	23	13.9	22.8	9.0	1,204	10.7
Standing start, 92 steps per minute							
III	63	3	8.6	11.8	13.7	1,370	7.7
VIII	60	2	5.6	5.7	13.2	1,618	9.7
IX	58	1	14.0	25.0	11.8	1,094	7.7
Average.....	60	6	9.4	14.2	12.9	1,361	8.4

By preliminary exercise such as walking or, indeed, stair-climbing the metabolism could artificially be brought to the level obtaining during the ascent. The nearer the metabolic level prior to the actual measurement reaches the true climbing level, obviously the more closely will the measured

metabolism represent the effort of ascent. Two different procedures were therefore followed in the stair-climbing experiments in Clapp Laboratory. In one series of experiments the subject began the ascent of the stairs after having stood quietly for several minutes, thus more nearly approximating the conditions obtaining in the ordinary house prior to the ascending of stairs but including the transitional period. In a second series (with the view of eliminating, in so far as possible, the effect of this complicating transitional period) the subject walked around the corridor for three or four minutes, usually at the rate of about 72 steps per minute, in order to begin the stair climbing with a somewhat elevated metabolism. Under these conditions, although the preliminary metabolism is certainly not brought, as it should be, to the level it attains in the ascent of stairs, at least it is considerably above the standing metabolism.

The average results obtained in these stair-climbing experiments are given in abstract in table 4. The significant values are the gram calories per vertical kilogrammeter, i.e., the total calories expended by the subject in raising one kilogram of body weight one meter in a vertical direction (including likewise the energy expended in living). These values are larger after the walking start than after the standing start, because in the latter case so large a proportion of the time of ascent was occupied by the period of transition. With the walking start the metabolic level had been raised somewhat, the effect of the transitional period had been lessened, and hence the energy measured during the climb more nearly represents that obtaining during true climbing, and a higher heat factor per vertical kilogrammeter is observed.

When the rate of walking was changed from 72 to 92 steps per minute (from a standing start), the gram calories per vertical kilogrammeter were lower in every case. This is explained by the fact that at the faster rate of stair-climbing the transitional period occupied an even greater proportion of the time of ascent, which was less than two minutes.

The obvious conclusion to be drawn from the study of the data given in table 4 is that the measurement of the effect of the transition from standing to walking is of fundamental importance in determining the total energy expenditure as a result of the stair-climbing. Recognizing that the transition exists, that the landings on the stairs introduce disturbing factors in the time relations in the climbing, and furthern ore that these landings would permit a certain recuperatory phase in the work of walking, we believe that it is nevertheless permissible to draw general conclusions, disregarding completely the period of transition at the beginning of the exercise. Thus, we note from table 4 that the total gram calories per vertical kilogrammeter after the standing start are on the average 8.8 at 72 steps per minute, and 8.4 at 92 steps per minute. After the walking start the average value is 10.7 calories. The total metabolism per hori-

zontal kilogrammeter was found to be in general 0.77 calorie. Hence when the individual ascends one meter, the total metabolism is approximately ten to thirteen times greater than when he walks horizontally one meter. But this will vary considerably, depending upon whether the subject has been standing or walking previous to the ascent.

In table 4, as already pointed out, the values after the standing start differ from those after the walking start in that in the latter case the initial metabolism prior to the ascent was much nearer the true climbing level. Prior to our long series of experiments on a continuous mountain stairway (see pages 689 to 695) still another method of securing the true increment in metabolism during stair-climbing was followed. As a result of the muscular effort of ascending stairs the total metabolic effect over and above the resting metabolism is the sum of the increment in oxygen consumption during the climbing period and the increment representing the higher level of metabolism sustained for some time after the climbing ceases, i.e., the after-effect (Benedict and Cathcart, 1913). Measurement of the increment only during the ascent fails to yield the total effect of the ascent. In some of the experiments carried out in the Clapp Laboratory after the standing start (unfortunately not in all) this after-effect was therefore measured, and in these cases it was possible to make an additive correction to the metabolism measured during the climb. This correction for the after-effect can of course be applied only to experiments made after a standing start and not after a walking start, since the metabolism after a walking start is probably already two or three times greater than the standing metabolism.

On several occasions the experiment made after the standing start (i.e., when there was no attempt in the experimental procedure to eliminate even partially the effect of the initial period of transition) was continued after the subject stopped walking, and the time required to consume about 750 cc. of oxygen was noted. The oxygen consumption immediately after work was frequently found to be even a little higher than it was on the average for the entire time of work, thus showing a pronounced after-effect. A variant of this type of experiment was made in that in some instances a few seconds after the stair-climbing experiment made from the standing start was ended, the subject was connected with a closed-circuit respiration apparatus and the oxygen consumption was measured for approximately five to six minutes, while the subject remained standing. Unquestionably at the end of this time the oxygen consumption had not reached the standing value noted prior to the walking, and the excess oxygen consumed above the standing value during this after-period represents the minimum rather than the maximum amount ascribable to the after-effect of the muscular effort.

A typical calculation based upon one such experiment made after the

standing start is as follows. With subject III during a period of standing after walking, the total excess oxygen measured over and above the initial standing value corresponded to 900 cc. Since the period of work was 2 minutes long, one can therefore assume that as an after-effect of the two minutes' work a minimum amount of 900 cc. of oxygen was consumed in excess of the resting value. Apportionment of this amount to the two minutes of work gives a correction of 450 cc. of oxygen to be applied to the per minute value measured during the work period. During the ascent period this subject consumed 1057 cc. of oxygen per minute. Her true oxygen consumption for the work of climbing the stairs was therefore 1500 cc. per minute. Multiplying this value by 4.825, the calorific value of oxygen at a respiratory quotient of 0.82,[5] and dividing the result by the vertical distance (10.65 meters) times the body weight (63 kgm.), we find that the calories per vertical kilogrammeter now reach a value of 10.8 instead of 8.1, the factor in which no correction for the after-effect is included. A similar calculation with another subject raised the value to 13.5. Unquestionably this type of correction, especially when the measurements of the after-effect are not carried further, is at best only an approximation. It does show clearly, however, that measurements during the period of climbing an ordinary stairway are noticeably low. If an average of two such divergent figures is permissible, these typical calculations indicate that the corrected factor for the total energy expended per vertical kilogrammeter in walking up the stairs in Clapp Laboratory would be on the average not far from 12.2. If such a correction were applied to all the values in table 4 determined after a standing start, the results would be nearer to the true values, but such correction is impossible.

Our efforts to secure a correction factor to apply to the measurement of the energy expended in the ascent of stairs, by measuring the after-effects of work, were admittedly only partially successful. They do, however, point out the difficulty of attempting to correlate the measurement of heat production following work with that during work. Not infrequently attempts have been made in research laboratories to approximate the metabolism during work, when the exercise was of such a character as to preclude the use of a respiration appliance during the work, by studying the metabolism immediately following the exertion (Govaerts, 1923; Viale, 1927). The extremely rapid transition in the metabolic plane following the change from work to rest is strikingly shown in the curves of Smith (1922), from which it is seen that inside of half a minute there is a tremendous change in the oxygen consumption and that unless connection with the respiration apparatus is made instantly after the cessation of work, a considerable amount of the after-effect is not measured.

[5] If one accepts the modern contention that the respiratory quotient of work is 1.00, this would alter the calculations by a small percentage, i.e., the calorific value of oxygen at a respiratory quotient of 1.00 is 5.047 instead of 4.825.

Nowhere has the measurement of the metabolism during work and immediately following work been more successfully carried out than in the researches of A. V. Hill and his associates (Hill, Long and Lupton, 1924b). Our own experiments convince us that there is still much to be done in studying the correlation between the metabolism as measured during a definite period after the completion of work with that measured, for example, during the last minute or two minutes of work. It is not inconceivable that a sufficiently close correlation may thus be found to make it possible to study the metabolism during many forms of muscular activity, which do not permit close attachment to a mouthpiece or helmet, by studying the oxygen consumption for one or more minutes immediately following work. Obviously in these cases graphic registration of the oxygen consumption is highly desirable. Hill and his associates were more particularly interested in accounting for the so-called "oxygen debt," but we wish to lay emphasis upon the relationship between the oxygen measurement in the period following work and the oxygen measurement during work as a possible means of predicting the latter from the former.

Respiration rates as affected by climbing. Although there were striking differences among the various subjects in the respiration rate (see table 4), both when the subjects were standing and when walking, the average picture with these young women shows an increment in rate of from 14 respirations per minute when standing to about 23 respirations when ascending stairs. No appreciable difference is to be observed, whether the subject ascended from a standing start or from a walking start.

One noticeable feature in table 4 is the extremely low respiration rate of subject VIII, which averaged 5.6 per minute when she was standing and which increased to less than 10 during the severe work of walking up several flights of stairs. It was noted frequently in a number of basal metabolism measurements with this subject that she had a respiration rate, when lying quietly, of not far from 3 per minute. Discovery of this extraordinary respiration rate led to some special experiments with subject VIII, in which the basal metabolism was determined and, incidentally, found to be well within normal limits. Electrocardiograms showed the sinus arrhythmia frequently occurring in normal people, and an x-ray showed that the diaphragm in normal respiration descended to about the same position as normally found with a forced inspiration. It was impossible to secure uniform respiratory quotients by any of several methods, and further studies are to be made with this young woman. For the purpose of this research it is only important to point out that experiments with this type of person were by no means easy to make, although they were successful.

Ascent of a mountain stairway. The troublesome effect of transition could be eliminated by a long preliminary period of climbing, possible only

on an escalator or an unbroken flight of steps such as paralleled the mountain railway at Mount Holyoke. Therefore, as soon as the weather permitted, experiments were made on the covered mountain stairway (522 steps; angle of ascent, 37°) on Mount Holyoke. The temperature ranged from 12° to 20°C. Each subject rested for a short interval after the climb and then walked to the foot of the steps for another experiment. The rate of ascent was regulated by a metronome and was purposely adjusted to about 45 steps per minute, to allow for the long climb. At this rate the total climb was made in about one and one-half or two minutes.

The length of time or the number of steps to be walked during the preliminary period was first tested, and approximately 130 steps were ascended before the first quantitative measurement, i.e., the use of the first bag of metered oxygen, was begun. When this oxygen was consumed, the stopcock was turned to the second bag and a second period of measurement immediately followed. Theoretically, these two periods should give essentially the same values, provided the subject had walked for a sufficiently long preliminary period to adjust her metabolism to the normal level required for walking at that rate and that elevation. With three subjects the oxygen consumption per stair during the second period varied from that during the first period in no regular manner, that is, it was as often above as below, showing that the preliminary period of walking 130 (occasionally 200) steps was sufficiently long to rule out all effect of the transition. The results for these two periods were therefore averaged in the case of these three subjects.

In one case (subject VIII) three experiments, made after a preliminary climb of about 150 stairs, showed that in the second period there was a considerably greater oxygen consumption per stair than in the first period. This was true in all three experiments and notably so in two of them, thus giving clear evidence that the metabolism was not adjusted to the work of climbing during the first experimental period. We do not know whether it was in the second period or not, except that the total oxygen consumption for the distance travelled was closely the same in the second period of all three experiments made with her, which would point toward a probable adjustment. Two complicating factors with subject VIII, however, are that her rate of walking was somewhat more rapid than with the other subjects and that, as previously discussed (see page 689), she exhibited in general an unusual rate and depth of respiration when standing as well as when walking. With this subject, therefore, the average of the results obtained in the second periods only is reported.

Twenty-five experimental periods in all were made on the mountain stairway with four subjects, the data for which are summarized in table 5. The values in the last column of this table were obtained by calculating from the total oxygen consumption the total energy expenditure and

dividing this by the body weight (including clothing and weight of apparatus) times the vertical distance climbed. This factor, i.e., the gram calories per vertical kilogrammeter of ascent, represents both the basal metabolism of the subject and the increment in metabolism due to the effort of climbing, expressed in relation to the body weight and the vertical distance climbed.

The average factor for all four subjects on this basis is 11.9, all the values lying reasonably close to this average. Theoretically, judging from experiments on walking on a level, differences in this factor depending upon the rate of ascent would be expected. Our data do not lend themselves to convincing discussion of this point, however, since an effort was made to have the rate of climbing fairly normal. In these tests the subjects had been performing rather severe work. They were not exhausted but were willing to stop, and it would seem as if this average value of 11.9 represents closely the

TABLE 5

Total metabolism during ascent of a mountain stairway

SUBJECT	WEIGHT AS CLIMBED	NUMBER OF EXPERIMENTAL PERIODS	STEPS ASCENDED PER MINUTE	VERTICAL METERS ASCENDED PER MINUTE	TOTAL O_2 CONSUMED PER MINUTE	GRAM CALORIES PER VERTICAL KILOGRAMMETER OF ASCENT
	kgm.				*cc.*	
I	70	4	45	9.5	1,652	12.0
XI	68	11	44	9.2	1,587	12.2
VIII	57	3	57	11.9	1,660	11.8
XII	52	7	46	9.6	1,180	11.4
Average	62	25	48	10.1	1,519	11.9

energy expended in raising one kilogram one vertical meter over a flight of stairs at an angle of 37 degrees. Since this angle of ascent is not far from that obtaining with ordinary stairs, this average factor could be used in computing the energy expended in ordinary stair climbing. Indeed, it lies near to 12.2, the average of the two computed values to which the correction for the after-effect was applied in the Clapp Laboratory experiments.

As a gross factor for practical use, therefore, 12 would represent the calories per vertical kilogrammeter when one is climbing stairs, and multiplication of the body weight in kilograms by the vertical distance travelled in meters and by the factor 12 would give the total caloric expenditure as a result of the climb, *including the resting metabolism for the actual time of the climb.*

It is difficult to compare this value for the work of ascent in stair climbing directly with that measured on an inclined plane, an inclined concrete

walk, or a belted treadmill, all of which have a smooth walking surface undoubtedly calling for much simpler incidental muscular movements. Stair climbing is, however, a part of each person's existence, and this factor has definite value in computing the energy involved in ascending stairs.

Since the standing metabolism of our subjects is reasonably well known and the work of forward progression, i.e., the horizontal component, is readily computed, it is perhaps of value to compute the increment in metabolism (over and above the standing metabolism and the work of forward progression) specifically ascribable to the work of vertical lift. By converting to heat the value for the total oxygen consumption as measured during the climbing experiment, deducting the heat required for standing and that required to move the weight of the body a definite distance in forward progression (obtained by multiplying the body weight by the horizontal distance walked in meters by the factor 0.51), we have the increment in heat expended per kilogrammeter of vertical lift, as follows:

SUBJECT	HEAT INCREMENT PER VERTICAL KILOGRAMMETER OF ASCENT
I	9.5
VIII	9.2
XI	9.7
XII	8.6
Average	9.3 gram calories

From the ratio between this average of 9.3 gram calories and the known calorie value of a vertical kilogrammeter (2.344 gram calories), the average mechanical efficiency of these young women has been computed to be 25 per cent, the values for the individual subjects all being close to this average. This regularity in the mechanical efficiency of our subjects is in contra-distinction to the findings of Waller (see page 676), which may perhaps be accounted for by the type of technique employed by him. Smith (1922) has listed comparable factors given by previous investigators, and the values all range somewhat higher than 25 per cent. The type of climbing, however, was treadmill walking at different grades and did not include staircase climbing, which involves a different set of muscular motions than walking on a smooth or, indeed, a mountain path. Here again, as in the case of the horizontal walking, it is perhaps striking that men and women with such different gaits should have a mechanical efficiency so close to each other.

Descent of a mountain stairway. The long, unbroken flight of stairs on Mount Holyoke presented ideal conditions for studying the work of descent. Furthermore, since the cable railway was in operation on one or

two days, the subjects could on these days ride up the mountain in a car and then descend without having to undergo the effort of walking up prior to the study of the descent. Experiments were carried out with four young women.[6] In some instances the subject had climbed about 300 steps for the ascent experiment and had then rested for from 8 to 11 minutes before beginning the descent. In others she rode to the top of the stairs in the cable car, and then descended about 150 preliminary steps before the actual measurements were made. Thus the transitional period preceded the measurement. The slow rate of climbing used in the ascent experiments was found difficult to maintain by some of the subjects in going downstairs. The metronome was therefore not used, but each girl was

TABLE 6

Total metabolism during descent of a mountain stairway

SUBJECT	WEIGHT AS DESCENDED	NUMBER OF EXPERIMENTAL PERIODS	STEPS DESCENDED PER MINUTE	VERTICAL METERS DESCENDED PER MINUTE	TOTAL O_2 CONSUMED PER MINUTE	GRAM CALORIES PER VERTICAL KILOGRAM-METER OF DESCENT
	kgm.				*cc.*	
I	70	5*	102	21.3	987	3.2
XI	68	2†	76	15.9	856	3.8
II	66	4*	73	15.2	785	3.8
VIII	57	5†	76	15.9	886	4.7
Average....	65	16	82	17.1	879	3.9

* Rode to the top of the stairs in a car.

† Climbed about 300 stairs for the ascent experiment and then rested 8 to 11 minutes before descending.

allowed to assume her natural rate in descent. This varied from 62 to 107 steps per minute. Here was obviously a technical error.

The total energy expenditure per vertical kilogrammeter of descent, uncorrected for the effort of standing and of forward progression, is recorded in table 6. With these four subjects the average factor on this basis is 3.9 calories.

[6] The results with these four subjects were reasonably uniform. With another young woman (subject XII) with whom in the ascent experiments results were obtained in full concordance with the results on other subjects and whose standing metabolism was normal, aberrant values were found when she descended the mountain stairway. No explanation is at hand for this. We have emphasized in the first part of this discussion that our technique lends itself to the rapid calculation of results. Greatly to our regret however, the descent experiment with subject XII was made at the end of the entire experimental series, and the aberrant results were noted too late to repeat the experiment with her. Further study of the work of descent is therefore desirable.

Unfortunately, we could not use the cable railway for the descent experiments as much as we had hoped. An examination of the data in table 6, however, shows that there was a tendency for those subjects who were carried up by the railway and then walked down to have a slightly lower energy expenditure per vertical kilogrammeter of descent than when they walked up, rested for about ten minutes, and then began the descent. The higher values found during the descent after walking up are perhaps to be expected, on the ground that the after-effect of the rather severe work of ascent had not passed off during the ten minutes of rest before the descent. Strictly speaking, comparisons should have been made more extensively with the same individual both after walking up and after going up in the cable car.

Although there is considerable irregularity in the final figures, the average of approximately 4 calories can be rationally compared to that of 12 for the work of ascent, from which it can be seen that the total energy expended in descent was about 33 per cent of that expended in ascent, or the energy involved in going down three flights of stairs about represents that expended in going up one flight.

A comparison of the total oxygen consumption per minute during horizontal walking with that during the descent of the mountain stairway, when the rates of walking were essentially the same (i.e., considering the total distance walked on the *incline* of the mountain stairway and not simply the distance represented by forward progression), indicates that with the two subjects for whom comparable data are available the total energy expenditure per minute during descent was 50 and 85 per cent greater than that during level walking.

The increment in the energy expenditure due to the work of descent, that is, deducting the standing metabolism and the energy expended in forward progression, has been computed to be as follows:

SUBJECT	HEAT INCREMENT PER VERTICAL KILOGRAMMETER OF DESCENT
I	1.7
II	1.6
VIII	2.5
XI	1.9
Average	1.9 gram calories

Comparison of the average increment of 1.9 calories with the average increment of 9.3 calories noted in the ascent experiments indicates that the *extra* effort required in going upstairs is about five times greater than that in going downstairs. In the act of descent heat is developed due to a

change in the energy of position of the body, but this is not the place to enter into a discussion of the purely physical side of this physiologically complex situation. It does, however, offer opportunity for many attractive speculations.

A comparison of the energy factors in the use of the staircase and their practical applications. From the standpoint of the physician wishing to prescribe either moderate or severe exercise for his patients or to counsel against exercise, a comparison in simplest terms of the energy expended in walking on the level and in going up and down stairs may be helpful. The physician's problem, for instance, might be to advise in the case of an adult of average weight (60 kgm. or 132 pounds, including clothing). The average staircase in the ordinary house has 15 steps in each flight, each having a vertical height of 20 cm. ($7\frac{3}{4}$ inches). The amount of energy expended by a 60-kgm. adult in walking up such a flight of stairs would be computed as follows: The total vertical distance ascended would be 3 meters (9.8 feet). Our experiments have shown that the total energy expended in lifting one kilogram (2.2 pounds) one vertical meter amounts to 12 gram calories.[7] Multiplication of this factor by 60 (the man's body weight in kilograms) and by 3 (the total meters ascended) gives 2160 gram calories as the total energy expended by the average individual in going up one flight of stairs. If this same amount of energy, i.e., 2160 calories were expended in walking on a level, the man or woman could walk 46.8 meters (154 feet). Thus, according to our horizontal walking experiments the factor per horizontal kilogrammeter is 0.77 calorie. A 60-kgm. adult would therefore expend 46.2 calories (0.77×60) in walking one horizontal meter, and to expend 2160 calories would have to walk 46.8 meters ($2160 \div 46.2$) or about fifteen times the distance represented in the ascent of 15 steps.

The energy expenditure per vertical kilogrammeter in the descent of stairs is 4 gram calories. The total expenditure in descending a flight of 15 steps or 3 meters would therefore be 720 gram calories ($4 \times 3 \times 60$). With this amount of energy the individual could walk on a level 15.5 meters (720 divided by 0.77×60) or about five times as far as on the staircase.

Similarly, three flights ($2160 \div 720$) of stairs could be descended with the same energy expenditure as in the ascent of one flight.

SUMMARY

With a respiration apparatus especially designed to be carried in larger part by the operator rather than by the subject, the oxygen consumption during horizontal walking and during the ascent and descent of stairs was

[7] It is here emphasized that throughout all this discussion our results are reported in gram calories. One thousand gram calories equal one large calorie.

studied with twelve young women at Mount Holyoke College, South Hadley, Massachusetts.

During horizontal walking at speeds of 34, 65 and 89 meters per minute the total heat production per horizontal kilogrammeter averaged 1.18, 0.79 and 0.87 gram calorie, respectively. The increment in heat production (i.e., deducting the energy required for standing) averaged 0.64, 0.52 and 0.67 calorie per horizontal kilogrammeter. These values indicate that the optimum rate of walking is at about 65 meters per minute and that sauntering is uneconomical.

During the descent of an ordinary flight of stairs *from a standing start*, at a rate of 72 to 92 steps per minute, the total energy expenditure averaged 8.8 and 8.4 gram calories, respectively, per vertical kilogrammeter. *After a walking start*, the average value was 10.7 calories. Since the total heat production per horizontal kilogrammeter averaged 0.79 calorie, it therefore costs approximately ten to thirteen times as much to ascend a vertical distance of one meter as to walk horizontally the same distance. A correction for the period of transition from the standing to the climbing level of metabolism was made in a few instances by measurement of the after-effect of the exercise. This correction raised the average total energy expenditure per vertical kilogrammeter to about 12.2 calories.

A mountain stairway, consisting of an unbroken flight of 522 steps, was subsequently available and thus enabled sufficient preliminary ascent (of at least 130 steps) to rule out the effect of the transitional period. The total energy expended per vertical kilogrammeter averaged in this series 12 gram calories, or fifteen times the energy expended per horizontal kilogrammeter in walking at the rate of about two or two and one-half miles per hour. The increment in heat production (i.e., deducting the energy involved in standing and in forward progression) amounted to 9.3 gram calories on the average. From this increment and the known calorie value of a vertical kilogrammeter the mechanical efficiency of these women in vertical lift was computed to be 25 per cent, surprisingly close to that found with men in spite of the different gaits of men and women.

Descent of the mountain stairway required on the average a total energy expenditure of 3.9 calories per vertical kilogrammeter. The total energy expended in descent was therefore about 33 per cent of that expended in ascent. The increment in heat due to the work of descent was 1.9 calories per vertical kilogrammeter.

For practical purposes it can be considered that the average person expends the same amount of energy in walking up one average flight of steps (15 steps, each 20 cm. high) as he does in walking on a level fifteen times the distance represented by the vertical height of such a staircase, or as he does in descending three such flights of steps. These comparisons may be helpful to the physician in prescribing exercise for patients.

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THE RELATION OF THE SHAPE OF THE ACTION POTENTIAL OF NERVE TO CONDUCTION VELOCITY

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The history of nerve physics is marked by speculation and experimentation directed toward the determination of the relationship between the various manifestations of nerve activity. After Bernstein had shown that the electrical wave is translated in nerve at the same rate as the wave of activity, attempts soon arose to place these two events in causal relationship. Du Bois-Reymond, speaking in terms of his molecular theory, said "die negative Schwankung ist vielleicht das Mittel, durch welches eine Querscheibe der Nervenfasern auf die Nächstfolgenden wirkt, ... Sie kann ebenso gut nur äusseres Merkmal der Molecularveränderung sein." At the conclusion of the present paper we will arrive at approximately the same opinion, namely, that the action potential *is* the sign of a local change and that it *may* furnish the energy for propagation. Hermann formulated the electrical theory in more detail. With the aid of a diagram he designated how the currents, which would be set up in the resting nerve substance by the active substance, would necessarily be stimulatory in nature. In the half century since Hermann, the idea has appeared in many places in the physiological literature, for instance in Cremer's (1909) core-conductor theory, and in its devlopment considerable importance has been attached to the time of the rise of the electrical disturbance in relation to the velocity of conduction. Lucas in 1909 made some accurate measurements on muscle by means of the capillary electrometer, which show that the two phenomena have the same temperature coefficient. The significance of these experiments is based on his reasoning that, "if it is the electrical disturbance in one part of a fiber's length which is the immediate cause of the same disturbance in the neighboring part, the rate at which the disturbance is propagated will depend upon the rate at which it develops in each section of the fiber's length."

In 1914 R. Lillie collected from the literature on various tissues data which show a general parallelism between the rate at which the action-current rises to maximum and the rate of propagation. Based on this and other considerations he evolved an elaborate argument in favor of the

electrical basis of conduction. In later papers he drew the analogy between local action in metals and protoplasmic transmission, finally arriving at his well-known iron wire model (1918), which has done more than anything else to support and visualize the electrical theory.

Due to the imperfections in the older measurements of nerve potentials the data which Lillie was forced to utilize in the case of nerve were not sufficiently accurate for the purpose, and therefore could not show anything more exact than that nerve, the tissue of fastest conduction, has the shortest rising phase. Accordingly, in view of the desirability of more accurate data for use in the argument as to the mechanism of the impulse translation in this tissue the present investigation was undertaken.

METHODS. In the first place, the data must be collected on fibers of the same size. While the duration of the rising phase of the action potential is the same in all the fibers of a nerve trunk, the velocities are very different, some being five times and more slower than others. This difference is not, however, as might seem at first sight, contradictory to the postulated relation between velocity and potential duration but is due (Lapicque and Legendre; Lapicque, Gasser and Desoille; Gasser and Erlanger) to differences in fiber structure. The velocity is a linear function of the diameter of the fibers.

The easiest way to get data on fibers of uniform size is to take the same fiber under different conditions, and since the fibers in a nerve trunk vary in size the best fibers to select are the largest ones, for the reason that their propagation velocity is the fastest and may therefore be determined by the interval between the artefact of the electrical stimulus and the beginning of the action potential, as recorded after conduction to a given distance from the stimulus. The obtaining of the potential wave form in such a fiber is more difficult. Obviously the form is not that recorded in a mixed nerve after conduction, as was assumed in the older experiments, on account of the temporal dispersion of the constituents. It can only be measured at the point of stimulation and then only because the duration is the same in all fibers, the recorded wave being the summation of similar constituents in phase. As much of the disturbing artefact, due to the stimulus, is caused by polarization of the sheath (Bishop, Erlanger and Gasser) the nerves selected for study were the motor roots of the bullfrog, which have no sheaths, and the phrenic nerve of the dog, which has a sheath which may be artificially removed. Both also have the advantage of being fairly homogeneous. The experiment was arranged as in figure 1. A pair of leads from the killed end and side of the nerve was connected to the cathode ray oscillograph. Two pairs of platinum electrodes were connected with an induction coil so that the stimulus could be either at *A*, for determination of the action potential form, or at *B*, to get the velocity of conduction. In the case of the bullfrog root the nerve was left attached

so that the conduction time could be more accurately determined. The stimulus was always supramaximal at the start of the experiment and left unchanged throughout its course, to insure that all fibers would be stimulated at all temperatures and that the shape of the artefact would be as nearly as possible the same at every observation. This was particularly essential where the height of the recorded wave was measured.

For the potential record even at the stimulus to reveal what is happening in any one fiber, it is necessary that the means used for altering the velocity of the nerve process be one which will act upon all fibers alike. This consideration eliminates at the outset all pharmacodynamic agents because these, as is well known, do not act equally on all fibers. Pressure acts even more differentially so temperature changes were selected as being most free from this objection. But even this agent has been shown by Howell, Budgett and Leonard to have a differential action on nerve fibers, and therefore a preliminary investigation of its extent was necessary. For this purpose a green-frog sciatic nerve was threaded through holes bored on opposite sides of an 8 mm. vulcanite tube. The action potential wave from a maximal stimulus was recorded at a distance from the stimulating electrode, so as to spread out the constituents. The nerve was then cooled to various temperatures at the portion traversing the cylinder, a procedure which would either block fibers altogether or transmit the impulses full size but delayed. Neither by the criterion of delay or block was there any significant differential action, and the experiment therefore justified the use of cold as a method. On the other hand, the observation should not be considered to be contradictory to Howell, Budgett and Leonard's results as the fibers in which they found the greatest differentiation would be small fibers and these contribute but little to the total area of the potential wave. Also this little is at the tail end of the conducted wave, so that even with an action of cold on the small fibers proportional to that on the large ones, the portion of the wave in which their potentials appear would be so spacially spread out that it would be impossible to determine the exact time of disappearance of individual constituents. In the present experiments on the form of the potential wave, the whole nerve was cooled by changing the temperature of the water in the jacket of the incubator in which the nerves were mounted; and photographs of the waves were made only after sufficient time had elapsed for full equilibrium. The hysteresis, sometimes seen on warming or cooling nerve, did not appear in these experiments.

Results. The observations in all experiments were uniformly consistent in showing that with the slowing of conduction the duration of the rising phase of the potential wave lengthened and the height decreased. The nature of the records from a phrenic nerve, made at three temperatures but under otherwise identical conditions, is shown in figure 2; and figure 3

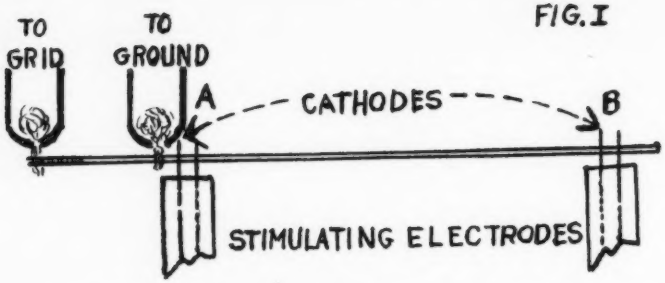


FIG. I

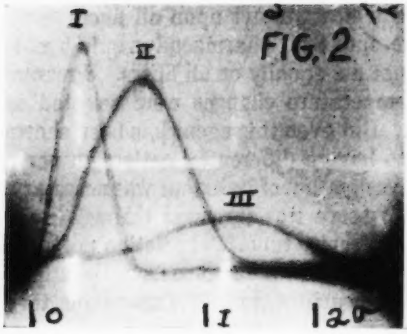


FIG. 2

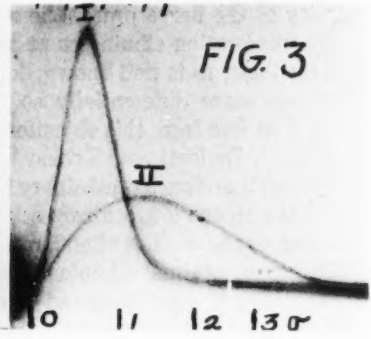


FIG. 3

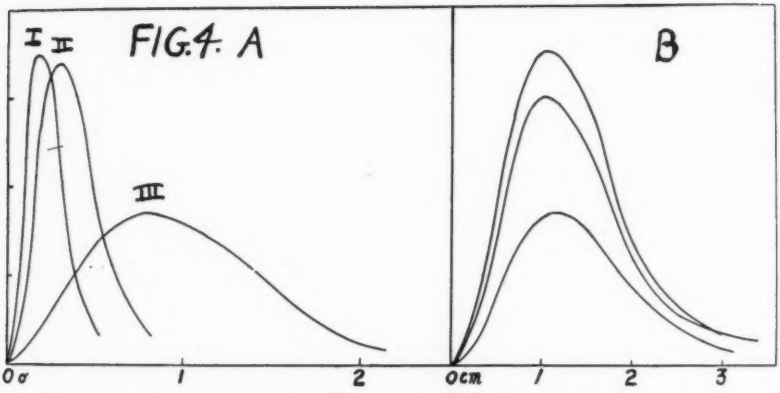


FIG. 4. A

B

Fig. 2. Dog phrenic (3/15/27). Print from three records superimposed according to the orientation (white) lines on the face of the tube, which brings record III slightly above the proper base line. Records retouched for publication. Stimulus at *A*. *I* at 37°, *II* at 25.6°, *III* at 19.2°. 1 mf. 2000$^{\omega}$ line, 48000$^{\omega}$ shunt.

Fig. 3. Bullfrog root (11/27/26). Stimulus at *A*. *I* at 24.2°, *II* at 12°. 1 mf. 5000$^{\omega}$ line, 32000$^{\omega}$ shunt.

Fig. 4. A—Potential-time curves from a phrenic nerve (3/22/27) drawn in rectangular linear coördinates. *I*, 37°; *II*, 25.4°; *III*, 18.6°. B—Potential-distance curves for the same data.

TABLE 1

		TEMPERATURE	VELOCITY	RISING PHASE	σi	MEASURED HEIGHT
Bullfrog motor roots						
			cm./σ	*σ*		*cm.*
12/ 3/26	12:28	22.7	4.22	0.51	2.14	
	2:35	11.6	1.84	1.12	2.07	
	3:30	22.9	3.83	0.55	2.10	
3/11/27	11:37	22.0	3.72	0.51	1.90	
	2:45	11.0	1.87	1.05	1.97	
	3:55	25.8	3.95	0.52	2.05	31.0
	4:15	25.0	3.88	0.47	1.82	41.0
	5:03	12.4	2.11	0.93	1.95	31.5
	5:30	25.0	3.92	0.45	1.76	40.0
10/25/27	11:05	26.3	4.50	0.52	2.34	37*
	11:26	16.0	2.17	1.10	2.39	28*
	11:43	11.8	1.74	1.47	2.56	20*
	12:11	11.7	1.35	1.78	2.41	16*
	1:20	22.9	3.55	0.67	2.38	
11/27/26	10:59	24.2	4.5	0.44	2.00	41.0
	11:40	13.0	2.18	0.90	1.96	17.8
	11:50	12.5	2.08	1.10	2.09	17.0
	12:20	12.0	1.97	1.08	2.13	15.0
	12:38	24.0	3.8	0.53	2.01	35.0
	12:43	24.7	4.0	0.51	2.04	37.0
	2:00	25.0	4.3	0.44	1.89	48.0
Dog phrenic						
11/23/26	4:10	37.7	5.68	0.23	1.30	43.0
	4:55	25.0	3.38	0.44	1.49	26.5
3/15/27	3:20	36.0	6.46	0.28	1.81	44.0
	3:55	25.6	4.00	0.48	1.92	30.0
	4:25	19.2	2.08	0.91	1.90	8.0
	4:52	37.0	6.62	0.28	1.86	38.0
3/18/27	12:20	37.2	5.81	0.18	1.03	38.0
	1:03	24.4	3.33	0.32	1.03	12.0
	2:10 †	18.5	1.82	0.67	1.22	16.0
3/22/27	1:15	37.0	5.87	0.18	1.06	35.0
	2:10	25.4	4.08	0.25	1.02	31.2
	3:00	18.6	1.82	0.77	1.02	16.0
	3:50	37.3	5.7	0.20	1.14	36.0

* A small but inexact correction for the artefact made in these measurements.
† This nerve returned to normal at 37° but was not recorded.

contains the records from a bullfrog at two temperatures. The potential-time curves from another phrenic nerve are redrawn in rectangular linear coördinates for figure 4A. The point of major interest is whether the rising phases are inversely proportional to the velocities of propagation. If this be so then the product of the velocity v and the time to maximum t should be a constant and a table of its values should show directly the degree of deviation from the postulated proportionality. These and other data appear in table 1. Furthermore the vt product gives at once the position of the crest in the active nerve, and since the tabulated products are close to a constant value it follows that the crests in a given fiber are a fixed distance behind the fronts at all velocities. This fact is in direct contrast to the condition in nerves whose different velocities are due to variation in the size of the fibers. Here the rising phases are constant while the velocities are variable and therefore the wave lengths are proportional to the latter and also to the diameters of the fibers. When the potential-distance curves are plotted at the various temperatures they are seen to differ in height only. Such curves appear in figure 4B which is calculated from the same data which gave figure 4A.

In searching for changes in the action potential having a simple relationship to the velocity of propagation the duration of the rising phase was the only one found. The slope of the rising phase of the potential-time curve has no relation to it whatever as one would expect from the inverse relation of v and t with a variable height. The magnitudes of the potentials and with them the slopes of the potential-distance curves decrease with temperature. The decrease, however, is not linear with respect to the velocity. On the other hand it does not deviate grossly from linearity. The question of the potential values will be taken up in a later section, though the data do not permit the establishment of the law of decrease.

The absolute potential in a nerve fiber is unknown; all we record is its relative values at different parts of the wave and these usually with a small error due to the residuum of artefact, when recording from the stimulus. But when we come to make comparisons between different temperatures we meet a new difficulty, the change in internal resistance of the fibers themselves with temperature, a change which has long been recognized (e.g., Bernstein, 1877). The conductivity of an electrolyte is known to alter about 2 per cent per degree Centigrade, and this degree of alteration was shown to hold for short ranges of temperature in nerve. To make resistance determinations the nerve was placed in one arm of a bridge, a thousand cycle approximately sinusoidal current was led through the bridge at a strength far below one that would stimulate a nerve. The bridge was balanced with the amplifier and oscillograph as a null instrument, a technique which is at once convenient and rapid. A green frog sciatic gave readings graphically shown in figure 6. In a desheathed

phrenic, the resistance was 60,100$^{\omega}$ at 37.7°, 76,000$^{\omega}$ at 25.8°, 100,500$^{\omega}$ at 16.8° and 66,800$^{\omega}$ on returning 37.7°. This resistance change with temperature has its effect upon the magnitude of the recorded potential. However, if the portions of the nerve acting respectively as source of potential and shunting resistance have the same temperature coefficient of resistance, the potentials recorded at different temperatures with a strictly potentiometric device would be proportional to the real potentials. Therefore the decrease from proportionality in the cooled nerves would be negligible if direct input to the amplifier were employed, because of the high input resistance. Unfortunately however the magnitude of the action potential is such that input shunts varying from 30,000 to 100,000 ohms were necessary to control the output potential to the proper height, and shunts of this value make the recorded potential wave dependent on the nerve's internal resistance. But, knowing the internal resistance of the nerve, the potential can be corrected to the value delivered at the surface of the nerve and magnitudes thus obtained of relative significance. In some experiments the resistance could be calculated from the heights of the records as made with two different shunts, but such data were not available in others as the experiments were planned to give time data rather than potential data. After the corrections are made we still find that there is definitely a large falling off in amplitude of the potentials in the cooled nerve, and moreover the amplitude seems to fall off less rapidly than the velocity decreases (fig. 7). This same relationship is also true in the other uncorrected experiments after allowance is made for the probable temperature correction.

Discussion. We are thus confronted with the problem of attempting to explain how, while conduction takes place inversely as the duration of the rising phase, this relationship can hold in spite of a variable height. To do so we must present our conception of the whole phenomenon.

Following its introduction by Lucas the term "propagated disturbance" is often used for the change that takes place in active irritable tissues. In his Croonian Lecture (1912) he defines the propagated disturbance as an unknown change which sweeps along the excitable tissue and considers that, according to then available evidence, the electrical response is a constant concomitant of it. Three years previously, in his paper in which he obtained evidence that the electrical disturbance is the basis of propagation of the excited state, in the stating of the problem he recalled the question of whether the electrical disturbance is itself propagated or is a local change called up at each point of the fiber's length by some other unknown propagated disturbance. As the electrical disturbance may be both local and propagated we feel that the definition requires further precision. A paper by Erlanger, Bishop and Gasser contains some experiments which indicate quite definitely that the potential recorded from nerve is essentially a sign of a local reaction. By the criterion that the refractory state starts with

local activity, it was found that the duration of the part of the potential wave which could represent anything else than local activity was of the order of 0.07σ. A greater precision was not possible due to the magnitude of the corrections with respect to the value measured. Therefore since the potential is mainly produced by a local change, the "propagated" electrical wave must be the sign of successively induced local reactions having their own time course, and this being the case we would like, in order to deviate as little as possible from existing usage, to conserve the term "propagated disturbance" for this successively induced local change of which the electrical sign is the best known one but of which there are certainly other signs as for example CO_2 production, and heat. Suggestions that the electrical disturbance *is* the propagated disturbance are directly contrary to this formulation, and if we wish to speak of electrical changes other than the potential sign of a local reaction, Lillie's expression "local bioelectric current" will very clearly serve. Suffice it to say that such branches of it as get into an external lead are probably very small.

It is convenient to speak of the *local reaction* independent of the fact of its successive induction. To do so without ambiguity it must be clearly differentiated from the "local excitatory process" of Lucas, which refers to the events which antecede the local reaction, for example, on the Nernst hypothesis, the salt concentration produced at an interface by a stimulating current. The local excitatory process depends on the nature of the external exciting force; the local reaction is the resulting change whose time course and intensity are determined by the local state; the propagated disturbance is this local reaction as it is successively induced. That the local reaction is chemical in character has been proposed at various times. In fact Hermann (1879) in speaking of his core model said, "The platinum wire with its liquid sheath is not a model of stimulatable nerve but only a model of its electrotonic properties. If one could have as core or sheath a substance which could be broken down by shocks and become galvanically active, then it would not be unthinkable that there would be artificially reproduced the excitation phenomena of nerve, that is the wave-like propagation of a galvanic phase." Such a model is essentially achieved in the iron wire model of R. Lillie, and the latter in his papers clearly defends the chemical element in transmission. The idea of a chemical change appears in the assimilation-disassimilation theory of Hering and has its analogue in the "physiological polarization" of Cremer (1909). It receives strong experimental support in the recent measurements of nerve metabolism by various observers, and this support is strengthened by the measurement of the heat production by Downing, Gerard and Hill.

The fact derived from column 5, table 1 and supported by analogous data on other tissues, that vt equals a constant, can be interpreted either as the result of the passage of a wave of constant length at different veloc-

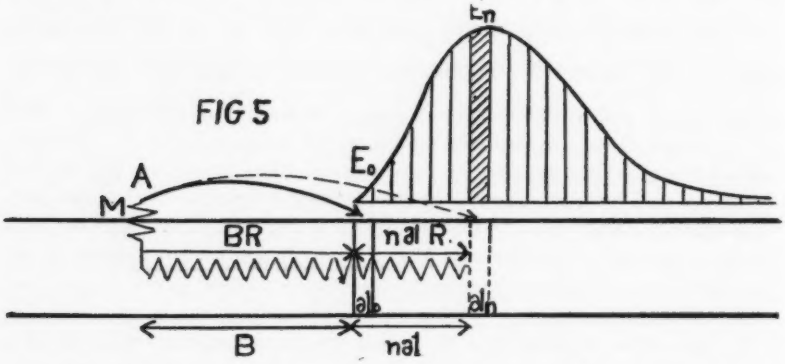
ities or as the potential-time curve of a process of different durations, which in some way determines a propagation rate inversely proportional to its duration. However since the former assumption would leave us entirely without ideas as to the origin of the wave or as to the causes of its velocity and wave length, it is necessary, as is usually done, to confine our attention to the much more probable second alternative.

The expression, vt equals a constant, may be written $v = C\frac{1}{t}$ and equations of this form are merely an expression of the observed experimental relationship. The factor of proportionality C depends upon a number of determining elements, some of which have been enumerated by Lillie, and it may be elaborated to indicate the nature of some of these elements. For instance the linear effect of fiber diameter, to which reference has already been made, may be included giving an expression $v = C'\frac{d}{t}$ where C' is a new proportionality factor. Similarly Lillie has derived the expression $v = K\frac{s}{t}$ where s is "the maximal distance at which the current between active and as yet inactive regions just suffices to stimulate." But since assumptions are possible which would indicate that the distance, ahead of an active segment of constant potential, at which the current density would have any specified value, is itself a function of the diameter, it is quite possible that the terms s and d of the two expressions are mutually dependent. In Cremer's formula (1922) many more constants are involved but in this case the velocity appears in relation to the square root of the rising phase.

In spite of the opinion that the action potential is an electrical sign of a local action the bioelectric circuit theory of Lillie may still be defended if we modify the value of s from that proposed by its author. From the distance apart at which two electrodes with a potential difference of 20 to 40 millivolts, which corresponds to a possible physiological one, could be made to stimulate a frog's nerve, he estimated the value of s as of the order of 3 cm. This estimation, drawn from conditions quite different from what must obtain in the postulated biological local circuit, we feel to be too large. The resistance of an axon which is the unit to which the theory must apply is very large. Its estimated value is around 10^9 ohms per centimeter depending on the size of the axon (Göthlin; Gasser and Erlanger). Therefore the current in the local circuit would fall off very rapidly ahead of the active region due to the effect of the axon resistance alone; for instance, with a potential of 1 volt the current 1 mm. away would be only 10^{-8} amperes. It seems probable therefore that the physiological local currents would become ineffective at a very short distance. Again, if we would reduce the conventional theoretical local bioelectric circuit

diagrams to nerve dimensions they would occupy only about 0.1 to 0.2 mm. of the axon's length. It may be for this reason that so little of the action potentials recorded by Erlanger, Bishop and Gasser was attributable to the local bioelectric circuits, and if so their experiments do not controvert the theory even if we accept only the lowest of their possible range of values.

After this introduction the discussion of the problem of the reduced potentials in the cooled nerves may be resumed. In the absence of a complete theoretical expression explaining the velocity of conduction we can turn the problem around and consider the stimulating value of the advancing wave making use of its mode of progression as defined by experimental data. The curves in part B, figure 4, show the distribution of potentials in the portions of a fiber which would be the source of the theoretical eddy currents in the inactive nerve. We may take some point A at a selected distance B ahead of the wave fronts (fig. 5). The eddy cur-



rents through it would be determined by the shape of the wave fronts, but as the latter are more or less symmetrical the currents would be proportional in magnitude to the heights of the waves. On the other hand these wave fronts are advancing on the point A at different velocities and as a result the currents through A would have different shapes and therefore different stimulating values.

The energy of the current through A during the period in which the wave is traversing the distance B must be just sufficient to start the local reaction in A at the arrival of the wave. To evaluate this energy let us divide the active part of the nerve into small segments δl wide (fig. 5). The first segment will be δl_o with a potential E_o, the succeeding segments will be described with the subscript n designating the number of the segment behind the first. The potentials E_n are values obtained from experiment and have no relation to the value of n, they are merely designated by a number. As they are the actual working potentials and not thermodynamic poten-

tials it is unnecessary to consider the effect of the external circuits upon them. To consider the current through A from the segment δl_n we may consider the resistance in the part of the circuit outside of the axon as O or as included in the axon, and the resistance across the membrane at δl_n as a constant, again for convenience giving the value O. The magnitude of the current will then depend upon the internal resistance plus the resistance M across the membrane at A. Let the axon resistance be R ohms per centimeter. The resistance of the circuit from δl_n through A will be $(B+n\delta l)\ R$. If the wave be traveling X centimeters per σ then the time for the wave to reach A will be B/X. The energy of the current during this period will be

$$W_n = \int_o^{B/X} E_n\, i_n\, dt \tag{1}$$

where i_n is the current. To integrate we must know E_n and i_n. E_n is a constant for any one segment; i_n depends on E_n and the instantaneous resistance which is $M + (B + n\delta l - Xt)\ R$. Substituting in (1) we obtain

$$W_n = \frac{E_n^2}{R} \int_o^{B/X} \frac{1}{\frac{M}{R} + B + n\delta l - Xt}\, dt \tag{2}$$

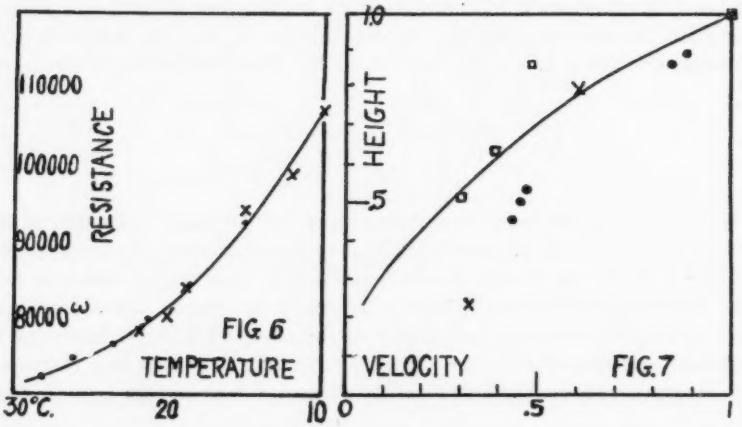
The total energy of the currents through A will be that of the sum of the currents from all the segments but due to the approximately symmetrical nature of the waves we are considering, the energy of the current from any one segment may be considered as typical of the whole. As a matter of fact the rapidly increasing resistance of the nerve with length leads one to suppose that the effect of the segment δl_n rapidly decreases as n increases.

Integrating (2) for the first segment where $n = o$ we obtain

$$W_o = \frac{E_o^2}{RX} \ln\left(1 + \frac{BR}{M}\right) \tag{3}$$

The above expression may be compared with the energy of stimulation currents applied from without, although this is not strictly justifiable. The time-strength curve of stimulation has a form which has not been exactly defined from a mathematical standpoint. However, as Lapicque (1926) points out, constant currents which stimulate at a sufficiently short duration have a constant energy. This follows directly from Nernst's expression $i\sqrt{t} = K$; $W = Ri^2t$, and is constant if the resistance R does not vary. Due to the high axon resistance the distance B over which the eddy currents would have any significant value would be short and therefore also the time B/X might be expected to fall in the period where Nernst's formula holds. If we regard R and M as constant then we

can calculate the expected potentials which would give a constant energy during the period that the disturbance is passing the fixed distance B at variable rates X. Assigning unit value to W, E and X for the highest value of X, such a curve, parabolic in form, is given for decreasing X values in figure 7, and with with it are plotted some measured potentials taken from table 1, column 6, but corrected for the internal resistance of the nerve. That the fall of potential is of the order of magnitude which one might theoretically expect is evident, but pressing the analysis beyond this point is unwarranted either by the assumptions or the data. The increased values of R, which in the experiments would go with the decreased value of X would depress the left end of the V–E curve of figure 7. Again, the assumption was also made that the energy necessary to start the local reaction is constant at all temperatures when actually it has been shown by Gotch and Macdonald and by Waller that



cooling the nerve increases the sensitivity to currents of long duration and decreases it to short ones; or, as Lapicque states it, cooling lowers the rheobase and prolongs chronaxie (p. 231). If we are dealing in the local bioelectric circuit with a stimulating current of short duration then the threshold would be raised as the nerve cools; however, it is possible that the energy necessary for stimulation would not increase rapidly enough to prevent the potential producing it from decreasing. The latter point might be tested experimentally but there is at the present time no series of data known to the author from which the calculation can be made. It would be desirable to know the energy minima for excitation at the two temperatures and the relative durations of the currents at which the minima obtain.

While the data on the relationship between the action potential and the

velocity of propagation are thus shown to be compatible with the theory of the local bioelectric circuit, strictly speaking they only show a relationship between the reaction of which the potential is the sign and the conduction velocity, and it is possible that any other form of energy freed in the reaction, provided it have the same time course, might serve the same function. For instance, the heat liberated in nerve activity represents a very appreciable amount of energy, as compared with the electrical energy (A. V. Hill); and this relationship also holds for the portion of the heat described as initial (Gerard). The probability that heat may be the stimulating agent is however reduced by the large amount of thermal energy stated to be necessary for nerve excitation.

SUMMARY

New data are collected bearing on the relationship of the shape of the axon action potential in nerve to the velocity of propagation.

It is found that the product of the duration of the rising phase of the potential wave and the velocity of conduction is essentially constant when the two variables are caused to alter with temperature.

The axon action potential decreases in magnitude in the cooled nerve.

The relation of these facts to theories of conduction is discussed.

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